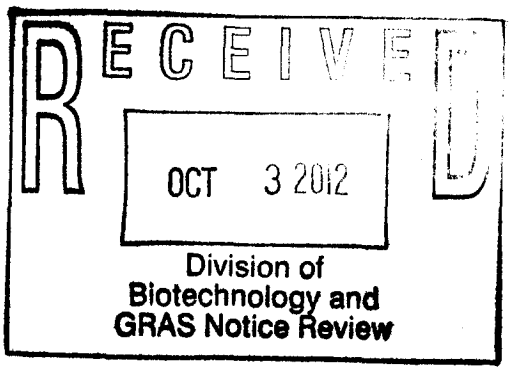
JHeimbach LLC



September 28, 2012

Paulette Gaynor, Ph.D.
Supervisory Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Paulette:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Groupe Grap'Sud, through me as its agent, hereby provides notice of a claim that the use of grape pomace extract as described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Groupe Grap'Sud has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, three copies of the notification, signed by the three members of the GRAS expert panel, are provided.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5548 or jh@jheimbach.com.

Sincerely,

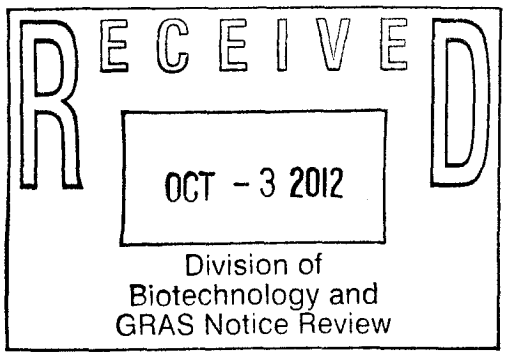
James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

923 Water Street, P.O. Box 66, Port Royal Virginia 22535, USA
tel. (+1) 804-742-5548 fax (+1) 202-478-0986 jh@jheimbach.com

Determination of the GRAS Status of the Addition of Grape Pomace Extract to Conventional Foods

Prepared for
Groupe Grap'Sud



Prepared by
JHeimbach LLC
Port Royal VA

July
2012

000003

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1. GRAS EXEMPTION CLAIM

Groupe Grap'Sud, through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration that the use of grape pomace extract described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Groupe Grap'Sud has determined through scientific procedures that such use of grape pomace extract is generally recognized as safe (GRAS).

[redacted]

James T. Heimbach, Ph.D., F.A.C.N.
President, JHEIMBACH LLC

9/28/12
Date

1.1. Name and Address of Notifier

Contact: Nelly Urban
Quality Manager, Research & Development
Groupe GRAP'SUD
30 360 Cruviers-Lascours
France

Telephone: 33 4 66 83 21 52

Facsimile: 33 4 66 83 22 70

E-mail: nurban@grapsud.com

1.2. Name of GRAS Substance

The substance that is the subject of this GRAS determination is red grape pomace extract. It is also known as grape extract, grape polyphenols, and by the brand name exGrape® Total. Grape pomace, like other fruit pomace, is the solid remains of the fruit after pressing for juice and contains pulp, skins, and seeds.

1.3. Intended Use and Consumer Exposure

Grape pomace extract is intended for addition to conventional foods as a source of antioxidants to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation (21 CFR §170.3[o]). The intended use of exGrape® Total grape pomace extract is identical to that of MegaNatural™ Gold grape seed extract (GSE) and grape skin extract (GSKE), which were the subjects of GRAS Notice No. 000125, submitted to FDA on February 21, 2003. (In its "no questions at this time" response on August 18, the FDA referred to GSKE as grape pomace extract or GPE.) These conditions of use include addition of exGrape® Total grape pomace extract to fruit juices for which no standard of identity exists, fruit-flavored beverages, fruit-flavored beverage mixes, and carbonated fruit-flavored beverages at a concentration of up to 210 parts per million.

In GRN 000125, the 90th percentile per-user intake of GSKE and GSE was estimated to be about 130 mg/day, equivalent to 4 mg/kg bw/day, based on Department of Agriculture survey data. Since the intended use of exGrape® Total grape pomace extract is identical to that of the existing MegaNatural™ products, the estimated daily intake of exGrape® Total is the same. Further, exGrape® Total grape pomace extract merely provides an alternative to the food

formulator, and so no additional consumer exposure to grape pomace extract will result from the use of the exGrape® Total brand

1.4. Basis for GRAS Determination

Determination of the safety and GRAS status of exGrape® Total grape pomace extract for direct addition to food under its intended conditions of use was made through the deliberations of an Expert Panel consisting of Joseph F. Borzelleca, Ph.D., Walter H. Glinsmann, M.D., and Robert J. Nicolosi, Ph.D., who reviewed the information in this monograph as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They critically reviewed and evaluated the publicly available information, including the potential human exposure to grape extract and intake of polyphenols and other constituents resulting from the intended use of exGrape® Total grape pomace extract, and individually and collectively concluded that the available information on grape pomace extract contains no evidence that demonstrates or suggests reasonable grounds to suspect a hazard to the public health under the intended conditions of use of exGrape® Total grape pomace extract.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion. Therefore, exGrape® Total grape pomace extract is GRAS by scientific procedures under the conditions of use described.

1.5. Availability of Information

The data and information that serve as the basis for the GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., President, JHEIMBACH LLC, 923 Water Street, P.O. Box 66, Port Royal VA 22535, telephone 804-742-5548 and e-mail jh@jheimbach.com.

1.6. Abbreviations

ALP: alkaline phosphatase
ALT: alanine aminotransferase
AST: aspartate aminotransferase
BUN: blood urea nitrogen
CFU: colony-forming units
cGMP: current good manufacturing practice
GC: gas chromatography
GLP: good laboratory practice
HDL: high-density lipoprotein
HPLC: high-performance liquid chromatography
INRA: Institut National de la Recherche Agronomique
LD_{50}: median lethal dose
LDL: low-density lipoprotein
MS: mass spectrometry
NCE: normochromatic erythrocytes

NOAEL: no observed adverse effect level
OPC: oligomeric proanthocyanidins
ORAC: oxygen radical absorbance capacity
PAC: proanthocyanidin
PCE: polychromatic erythrocytes
SCID: severe combined immunodeficient
TAC: total antioxidant capacity
TC: total cholesterol
TG: triacylglycerol
UPLC: ultra-performance liquid chromatography
VLDL: very low density lipoprotein

2. IDENTITY OF THE SUBSTANCE

2.1. Substance Name

The name of the substance is red grape pomace extract. Grape pomace, like other fruit pomace, is the solid remains of the fruit after pressing for juice and contains pulp, skins, and seeds.

2.2. Trade or Common Names

The red grape pomace extract is sold under the trade name exGrape® Total grape pomace extract. It is also known as PPR Exgrape®TOTAL, grape extract, grape polyphenols, and polyphenols from grape.

2.3. Botanical Identification

Grapes are the fruit of a perennial deciduous vine (*Vitis vinifera* L.) that is indigenous to southern Europe and western Asia, and which is cultivated today in all temperate regions of the world (Nassiri-Asl and Hosseinzadeh 2009). The color of grapes of different cultivars ranges from very dark nearly black purple to very light green, and are most often classified as red or white (Walker et al 2007). The color of red grapes is principally due to anthocyanins and other polyphenols; these substances are absent in white grapes due to mutations in two adjacent genes, VvMYBA1 and VvMYBA2, which remove the ability of the regulator to switch on anthocyanin biosynthesis (Walker et al. 2007). ExGrape® Total grape pomace extract is derived from red grapes.

2.4. Manufacturing Process

The manufacturing process is outlined in the flowchart in Figure 1. All processes are performed in compliance with ISO 9001, equivalent to cGMP.

After harvesting, sorting, cleaning, and removal of stems, red grapes are pressed to extract the juice, which is processed into wine. The remaining material, including pulp, skin, and seeds, constitutes grape pomace. Water is added to the pomace and maintained at a temperature of 35-70°C for approximately 3 hours, allowing water-soluble substances to be extracted into the aqueous juice of diffusion.

The juice, slightly fermented by the presence of naturally occurring *Saccharomyces* strains, is centrifuged and filtered to remove suspended particulate matter and is then passed through a column packed with adsorbent resin (styrene-divinylbenzene copolymer compliant with 21 CFR §173.65). Polyphenols that are adsorbed by the resin are recovered with an aqueous solution of food-grade ethyl alcohol. The eluate is vacuum dried under a temperature rising from 45°C to 75°C, removing the ethanol from the product, and pasteurized by raising the temperature to 95°C for 10 seconds. Residual ethanol does not exceed 100 mg/kg.

Finally, the concentrate is spray-dried and packed.

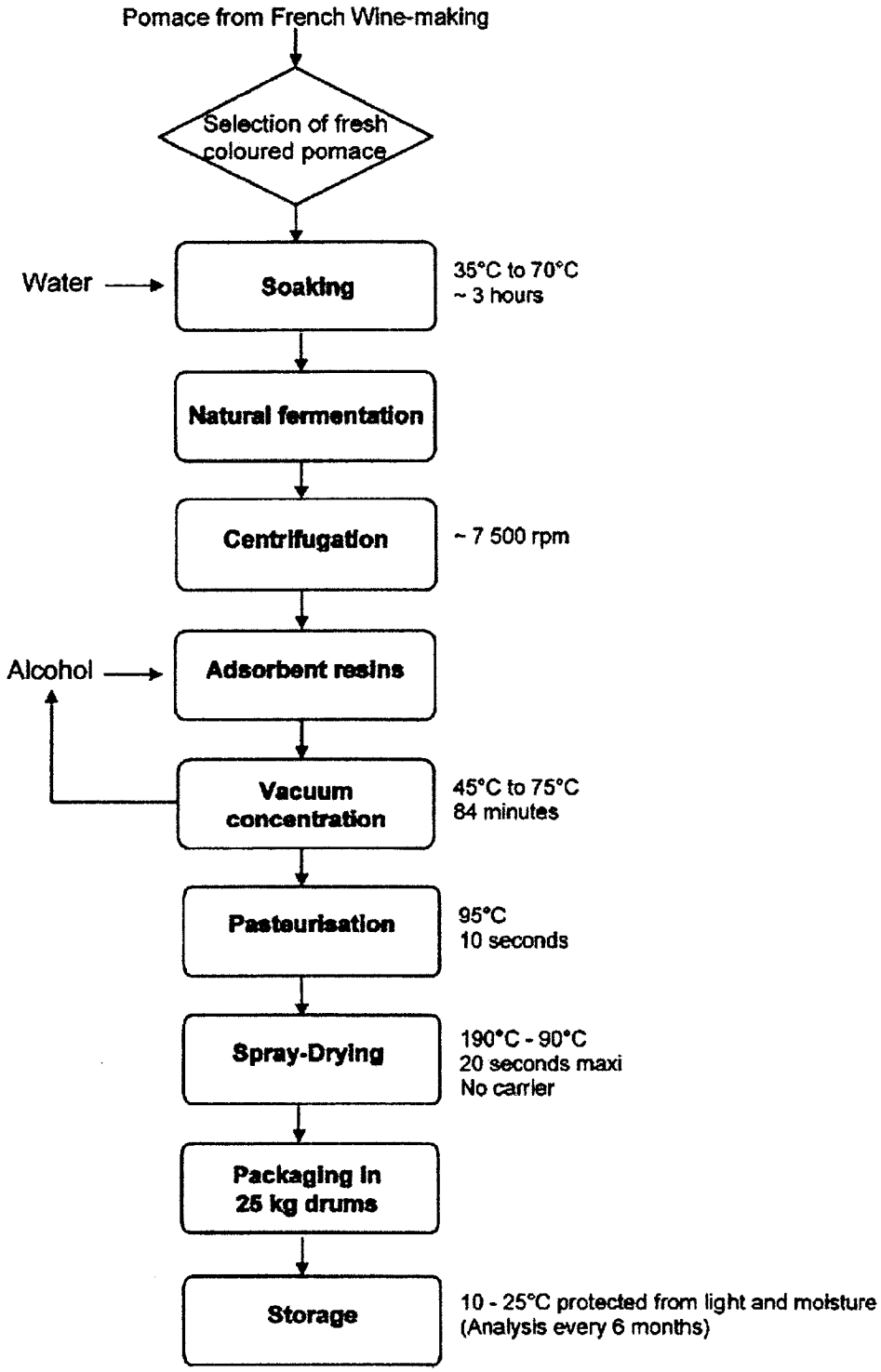

Figure 1. Production Process for exGrape® Total Grape Pomace Extract.

2.5. Physical and Chemical Properties

A brief summary of the physical and chemical properties of exGrape® Total grape pomace extract is provided in Table 1.

Table 1. Physical Characteristics of exGrape® Total Grape Pomace Extract.

Physical Standard	Value
Appearance	Fine powder
Particle sizes	100% < 0.15 mm
Color	Purple
Odor	Characteristic grape
Taste	Astringent
Bulk density	NLT[1] 0.40
Moisture	NMT[2] 7%
ORAC[3]	NLT 10,000 µmol TE/g

1. Not less than
2. Not more than
3. Measured in Trolox Equivalents (TE)

2.6. Composition of Grape Pomace Extract

The phenolic composition of one sample of exGrape® Total grape pomace extract, shown in Table 2, was published by Al-Awwadi et al. (2005). This analysis of a single sample is reported for completeness since it was published in the open literature, but it must be recognized that there is considerable sample-to-sample variability and the results of analysis of a single sample may reveal little about the range of phenolic content to be found in the product. The data presented in Table 3 are newer and more comprehensive.

Table 2. Phenolic Composition of exGrape® Total Grape Pomace Extract (Al-Awwadi et al. 2005).

Phenolic Compound	Content (mg/g powder)
Anthocyanins	
Delphinidin	113.2
Malvidin-3-glucoside	37.7
Peonidin	8.5
Cyanidin-3-glucoside	7.9
Cyanidin	3.9
trans-piceid	0.4
Procyanidins	
Dimer B1	59.1
Epicatechin	45.3
Gallic acid	8.3
Viniferin	2.1
cis-resveratrol	0.5

More recently, analyses were performed on 5 lots of exGrape® Total grape pomace extract by the Institut National de la Recherche Agronomique (INRA). In the first set of analyses, samples were analyzed using ultraviolet-visible spectroscopy with wavelengths of 280, 320, 360, and 520 nm. The results are shown in Table 3 and the inherent variability of phenolic content is evident.

Table 3. Polyphenolic Content of Five Lots of exGrape® Total Grape Pomace Extract.

Family	Compound	Lot				
		L09145 (mg/g)	L09302 (mg/g)	L09343 (mg/g)	L10061 (mg/g)	L11073 (mg/g)
Gallic acid and derived	Gallic acid	0.91	0.84	0.78	0.92	1.00
	Ethyl gallate	0.31	1.22	1.93	1.54	1.01
	Total	**1.22**	**2.07**	**2.72**	**2.46**	**2.01**
Flavan-3-ols	Catechin	11.47	13.52	11.99	12.91	6.13
	Epicatechin	9.84	12.71	16.12	18.30	10.31
	Dimer B1	10.28	10.77	11.38	11.74	8.30
	Dimer B2	7.17	7.85	7.98	8.76	6.79
	Dimer B3	0.65	0.90	0.77	0.99	0.64
	Dimer B4	1.82	1.33	1.31	1.52	1.27
	Dimer galloyled	1.33	1.38	3.73	1.93	1.62
	Dimer (epi)cat-epigallocatechin	0.40	0.27	0.31	0.32	0.21
	Trimer	2.05	1.46	1.93	2.10	1.28
	Total	**45.01**	**50.42**	**55.52**	**58.57**	**36.55**
Stilbens	*Trans*-resveratrol	0.29	0.33	0.30	0.19	0.16
	Ε-viniferin	0.10	0.07	0.08	0.09	0.05
	Cis-resveratrol	0.30	0.38	0.43	0.40	0.35
	Total	**0.70**	**0.78**	**0.81**	**0.68**	**0.56**
Dihydro-flavonol	Astilbin	**0.82**	**0.39**	**0.58**	**0.58**	**0.51**
Hydroxy-cinnamic acids	Caftaric acid	1.87	0.13	0.19	0.11	0.07
	Cis-coutaric acid	0.20	ND	ND	ND	ND
	Trans-coutaric acid	0.88	0.09	0.16	0.05	0.02
	Caffeic acid	0.35	0.31	0.54	0.20	0.17
	Fertaric acid	0.22	0.40	1.49	0.62	0.42
	Coumaric acid	0.82	1.38	1.21	0.61	0.48
	Total	**4.34**	**2.32**	**3.62**	**1.59**	**1.16**
Flavonols	Myricetin 3-*O*-glucuronide	0.27	0.16	0.14	0.09	0.09
	Myricetin 3-*O*-glucoside	0.63	1.93	1.78	0.71	0.84
	Quercetin 3-*O*-galactoside	0.13	0.40	0.25	0.13	0.14
	Quercetin 3-*O*-glucuronide	3.95	8.70	4.49	3.05	3.81
	Quercetin 3-*O*-glucoside	0.61	4.22	2.62	1.13	1.40
	Laricitrin 3-*O*-glucoside	0.38	1.07	0.91	0.40	0.37
	Myricetin aglycone	0.25	0.38	0.36	0.50	0.23
	Syringetin glucoside	0.70	2.26	2.00	0.98	0.97
	Quercetin aglycone	1.17	2.17	2.55	1.75	1.11
	Total	**8.08**	**21.30**	**15.10**	**8.74**	**8.97**
Antho-cyanins	(Epi)catechin-malvidin 3-O-glucoside	0.23	0.08	0.07	0.05	0.07
	Delphinidin 3-O-glucoside	1.07	0.15	0.17	0.10	0.20
	Cyanidin 3-O-glucoside	0.24	0.03	0.06	0.04	0.05
	Peunidin 3-O-glucoside	1.22	0.18	0.19	0.11	0.21
	(Malvidin+peonidin) 3-O-glucoside	9.76	2.25	1.89	0.89	1.69
	Pyranomalvidin pyruvic	0.23	0.26	0.30	0.23	0.28
	Malvidin-ethyl-(epi)catechin	0.34	0.19	0.30	0.13	0.15
	(Malvidin+peonidin) acetyl glucoside	1.12	0.49	0.38	0.18	0.33
	Pyranomalvidin 3-O-coumaroyl glucoside	0.20	0.13	0.10	0.06	0.08
	Petunidin 3-O-coumaroyl glucoside	0.13	0.07	0.07	0.03	0.06
	(Malvidin+peonidin) 3-O-coumaroyl glucoside	0.69	0.23	0.21	0.10	0.19
	Pyranomalvidin vinylcatechol	ND	0.20	0.15	0.11	0.13
	Pyranomalvidin vinyl phenol	ND	0.06	0.02	0.02	0.03
	Total	**15.23**	**4.33**	**3.89**	**2.05**	**3.47**
TOTAL		**75.39**	**81.60**	**82.24**	**74.67**	**53.22**

Source: INRA.

In a second analysis, INRA depolymerized the proanthocyanidins in the presence of phloroglucinol and analyzed the constituent units by UPLC. The results of these analyses, which include the proanthocyanidin content, the mean degree of polymerization (i.e., the average number of monomers released by depolymerization), and the monomeric elements, are shown in Table 4.

Table 4. Post-Depolymerization Analysis of Proanthocyanidins in Five Lots of exGrape® Total Grape Pomace Extract.

Parameter	Lot				
	L09145	L09302	L09343	L10061	L11073
Content (mg/g)	337.95	289.20	315.60	300.53	329.99
Mean degree of polymerization	4.08	2.80	2.67	2.92	3.86
Epicatechin (%)	75.09	74.36	73.93	74.99	76.62
Catechin (%)	12.89	15.81	14.99	15.28	11.22
Epigallocatechin (%)	7.98	5.77	6.19	6.77	7.31
Epicatechin 3-*O*-gallate (%)	4.04	4.06	4.89	2.96	4.85
Source: INRA.					

Finally, INRA prepared a summary table (Table 5) combining the results of the two sets of analyses.

Table 5. Summary Table of Polyphenols in Five Lots of exGrape® Total Grape Pomace Extract.

Family	Lot				
	L09145	L09302	L09343	L10061	L11073
Hydroxybenzoic acids (mg/g)	1.22	2.04	2.72	2.46	2.01
Hydroxycinnamic acids (mg/g)	4.34	2.32	3.62	1.59	1.16
Flavonols (mg/g)	8.08	21.30	15.10	8.74	8.97
Dihydroflavonols (mg/g)	0.82	0.39	0.58	0.58	0.51
Stilbens (mg/g)	0.70	0.78	0.81	0.68	0.56
Anthocyanins (mg/g)	15.23	4.33	3.89	2.05	3.47
Flavan-3-ols (mg/g)	337.95	289.20	315.60	300.53	329.99
Total (mg/g)	**368.34**	**320.39**	**342.32**	**316.63**	**346.67**
Source: INRA.					

It is instructive to compare the phenolic content of exGrape® Total grape pomace extract with that of the two MegaNatural™ products that were the subjects of GRN125—grape seed extract (GSE) and grape skin extract (GSKE), which FDA referred to as grape pomace extract (GPE) in its response letter. In Table 6, the values reported for exGrape® Total are the means of the analytical findings for the five lots assessed by INRA while those for the two MegaNatural™ products are taken from Table 3.3 of GRN125. While there are substantial differences in the concentrations of specific polyphenols, the overall patterns are similar. All three products have proanthocyanidins as the major phenolic component, with over half of the proanthocyanidins in the form of oligomers and gallates, and monomeric flavanols as the next most prevalent species.

In all three products, the content of anthocyanins, quercetin, kaempferol, myricetin, and non-flavonoid polyphenols is relatively small.

Table 6. Phenolic Contents of exGrape® Total and MegaNatural™ GSE and GSKE.

Phenolic Compound	Content in Product (Dry Basis; % w/w)		
	exGrape® Total	MegaNatural™ GSE	MegaNatural™ GSKE
Total phenols (GAE)	73.42	95.17	94.57
Proanthocyanidins	49.21	81.78	80.20
Dimers & gallates	22.44	5.20	4.02
Trimers & gallates	1.76	1.95	2.41
Oligomers & gallates	25.01	74.63	73.77
Monomeric flavanols	24.66	7.41	5.53
Catechin	11.20	2.92	2.64
Epicatechin	13.46	4.32	2.81
Gallate	--[1]	0.17	0.08
Anthocyanins	5.79	--	1.68
Quercetin	1.75	0.00	0.23
Kaempferol	--	0.00	0.02
Myricetin	0.34	0.00	0.01
Non-flavonoid polyphenols	--	5.98	6.90

SOURCES: exGrape® Total—INRA; MegaNatural™ products—GRN125, Table 3.3

1. Not reported.

2.7. Specifications for Food-Grade Material

Grap'Sud has established specifications to assure food grade product; these specifications are listed in Table 7. The results of analyses of 5 lots of product are displayed in Table 8, showing that the production process is in control and consistently results in product meeting the established food-grade specifications.

Table 7. Specifications for exGrape® Total Grape Pomace Extract.

Parameter	Value	Method
Appearance	Fine powder	Visual inspection
Particle sizes	100% through #40 mesh	Sieving
Color	Purple	Visual inspection
Odor	Characteristic grape	Sensory inspection
Taste	Astringent	Sensory inspection
Bulk density	NLT[1] 0.40	Densimeter
Moisture (%)	NMT[2] 6.0%	IR balance
Total polyphenols as catechin equivalent (%)	NLT 92.0%	OD 280 nm
Total polyphenols as gallic acid equivalent (%)	NLT 55.0%	Folin method
Procyanidins (OPC) as catechin equivalent (%)	NLT 15%	Vanillin method
Anthocyanins (%)	NLT 2.0%	Ribereau-Gayon method
Resveratrol as *trans*-resveratrol equivalent (mg/kg)	NLT 100	HPLC
Residual ethanol (mg/kg)	NMT 100	GC
Microbiological purity		
Total viable aerobes (cfu[3]/g)	NMT 1000	Plate count agar
Yeasts and molds (cfu/g)	NMT 100	Yeast extract glucose chloramphenicol agar
Total coliforms (in 1 g)	None	Bouillon lactosé bilié au vert brillant
Escherichia coli (in 10 g)	None	NF V 08-053
Salmonella spp. (in 10 g)	None	NF EN ISO 6579
Heavy metals		
Lead (mg/kg)	NMT 2	AAS[4]
Arsenic (mg/kg)	NMT 3	AAS
Mercury (mg/kg)	NMT 1	AAS
Cadmium (mg/kg)	NMT 1	AAS
Pesticide residues		
Conformance with US Pharmacopoeia 561	Conform	GC-MS

1. Not less than
2. Not more than
3. Colony-forming units
4. Atomic absorption spectrophotometer

Table 8. Analyses of Five Lots of exGrape® Total Grape Pomace Extract.

Parameter	Specification	Lot				
		L09 287	L10 061	L09 343	L11 073	L10 060
Bulk density	NLT[1] 0.40	0.54	0.43	0.50	0.45	0.44
Moisture (%)	NMT[2] 6.0%	4.2	3.8	4.6	4.1	6.6
Total polyphenols as catechin equivalent[3] (%)	NLT 92.0%	97.4	93.2	131.0[3]	119.6[3]	101.1[3]
Total polyphenols as gallic acid equivalent (%)	NLT 55.0%	66.7	73.7	70.1	67.1	66.1
Procyanidins (OPC) as catechin equivalent (%)	NLT 15%	27.3	19.3	25.8	20.1	21.0
Anthocyanins (%)	NLT 2.0%	2.1	2.0	2.4	2.1	2.2
Resveratrol as *trans*-resveratrol equivalent (mg/g)	NLT 100	155.7	330.9	343.9	211.0	195.0
Residual ethanol (mg/kg)	NMT 100	32	27	74	32	16
Microbiological purity						
Total aerobes (cfu[4]/g)	NMT 1000	60	550	37	0	337
Yeasts and molds (cfu/g)	NMT 100	40	0	10	0	0
Total coliforms (in 1 g)	None	None	None	None	None	None
Escherichia coli (in 10 g)	None	None	None	None	None	None
Salmonella spp. (in 10 g)	None	None	None	None	None	None
Heavy metals						
Lead (mg/kg)	NMT 2	0.1	0.3	ND	ND	0.6
Arsenic (mg/kg)	NMT 3	0.04	0.1	0.1	0.4	0.1
Mercury (mg/kg)	NMT 1	0.02	0.06	0.06	0.01	0.09
Cadmium (mg/kg)	NMT 1	0.3	0.07	ND	0.09	0.06
Pesticide residues						
Conforms with US Pharmacopoeia (USP) 561	Conform	Conform	Conform	Conform	Conform	Conform

1. Not less than
2. Not more than
3. Catechin-equivalent values are based on a reference curve for catechin; since polyphenols absorb more at 280 nm than does catechin the value can exceed 100%.
4. Colony-forming units

In Table 9, specifications for exGrape® Total are compared with those for two other GRAS grape extracts, grape seed extract (with less than 5.5% catechin monomers) and MegaNatural™ Grape Skin Extract, the subjects of GRN124 and GRN125, respectively. While the specification for the minimum concentration of total polyphenols (as gallic acid equivalents) is lower for exGrape® Total than for the other two products, most other specifications established for the three products—especially those regarding impurities and contaminants—are similar, indicating that food formulators have freedom to select among the products and may regard them as substantially interchangeable.

Table 9. Specifications for exGrape® Total and Two Other GRAS Grape Extracts.

Parameter	exGrape Extract	GSE[1]	MegaNatural™ GSKE*
Appearance	Fine purple powder	No specification	Red purple powder
Particle sizes	100% through #40 mesh	No specification	0% through #35 mesh NMT[3] 20% through #80 NLT[4] 80% through #200
Bulk density	NLT 0.40	No specification	No specification
Moisture (%)	NMT 6.0%	NMT 8%	NMT 8.0%
Total polyphenols as catechin equivalent (%)	NLT 92.0%	No specification	No specification
Total polyphenols as gallic acid equivalent (%)	NLT 55.0%	NLT 78%	NLT 80%
LC-MS phenol profile	No specification	NMT 5.5% monomers	NLT 5% monomers 60-80% oligomers NMT 25% polymers
Procyanidins (OPC) as catechin equivalent (%)	NLT 15%	No specification	No specification
Anthocyanins (%)	NLT 2.0%	No specification	NLT 1.5%
Resveratrol as *trans*-resveratrol equivalent (mg/kg)	NLT 100	No specification	No specification
Residual ethanol (mg/kg)	NMT 100	No specification	No specification
Protein (%)	No specification	NMT 7.0	No specification
Ash (%)	No specification	NMT 1.0	No specification
Fat (%)	No specification	NMT 1.0	No specification
Polysaccharides (%)	No specification	NMT 12	No specification
Microbiological purity			
Total viable aerobes (cfu[5]/g)	NMT 1000	NMT 1000	NMT 1000
Yeasts and molds (cfu/g)	NMT 100	NMT 100	NMT 100
Total coliforms (cfu)	None in 1 g	NMT 3	NMT 10
Escherichia coli (cfu)	None in 10 g	NMT 3	None in 10 g
Salmonella spp (cfu)	None in 10 g	None	None in 30 g
Staphylococcus aureus (cfu)	No specification	NMT 10	No specification
Heavy metals			
Lead (mg/kg)	NMT 2	NMT 1.0	No specification
Arsenic (mg/kg)	NMT 3	NMT 5	No specification
Mercury (mg/kg)	NMT 1	NMT 0.20	No specification
Cadmium (mg/kg)	NMT 1	NMT 1.0	No specification
Pesticide residues			
Conformance with US Pharmacopoeia 561	Conform	No specification	8 pesticides & MRL[6]

1. SOURCE: GRN124, Table 3 (p. 9)
2. SOURCE: GRN125, Table 3.5 (p. 40)
3. Not more than
4. Not less than
5. Colony-forming units
6. Maximum residue levels

2.8. Contaminants

2.8.1. Mycotoxins

Three lots of grape pomace extract were analyzed for the presence of ochratoxin A using the HPLC-based Association Francaise de Normalisation (AFNOR) method NF EN 14133. Recoveries were 4.7, 4.5, and 1.9 µg/kg. No standard for maximum allowable ochratoxin A levels in grape pomace extract exists in the U.S. or elsewhere; the most comparable standard is the European Commission limit for dried vine fruits, which was set at 10.0 µg/kg (Commission Regulation No. 123/2005, 26 January 2005.)

2.8.2. Pesticide Residues

Two lots of grape pomace extract were analyzed for pesticide residues and metabolites. Only a small number of pesticide residues reached detection thresholds, and none was present at levels violative of the maximum residue levels listed in USP 561, "Articles of Botanical Origin" (USP 2012). The reports of the two analyses are in the Appendix.

2.9. Stability

The stability of samples from one lot of exGrape® Total grape pomace extract was tested at a pH range of 1 to 7, and at temperatures up to 100°C, for periods ranging from 5 minutes to 90 days. In the first experiment, one sample of the powder was stored in an incubator at pH 2.7 for 1 hour at a temperature of 100°C and another for 90 days at a temperature of 40°C. The results are shown in Table 10.

Table 10. Stability of exGrape® Total Grape Pomace Extract Powder.

Parameter	Initial Value (%)	1 Hour at 100°C (%)	90 Days at 40°C (%)
Total polyphenols (OD[1] at 280 nm)	100.60	97.93	106.90
Anthocyanins	6.48	7.13	5.80
Total monomers	4.20	4.62	4.85
Catechin	2.00	2.10	2.13
Epicatechin	1.98	1.82	2.17
Epigallocatechin	0.22	0.70	0.55
Dimer B1	1.53	2.59	1.92
Dimer B2	1.74	1.60	1.60
Procyanidin polymers	32.10	25.50	28.48
Total polyphenols (HPLC)	45.66	41.60	45.77
1. Optical density			

There was no evidence of significant degradation of the product under the conditions tested; all observed values showed only normal analytical variability.

Additional stability testing was performed with other samples from the same lot dissolved in distilled water to produce a 2% aqueous solution; single samples were stored for 5 minutes at 100°C, for 15 minutes at 60°C, or for 24 hours at 20°C. Results are shown in Table 11.

Table 11. Stability of exGrape® Total Grape Pomace Extract at Different Temperatures.

Parameter	Initial Value (%)	5 Minutes at 100°C (%)	15 Minutes at 60°C (%)	24 Hours at 20°C (%)
Total polyphenols (OD[1] at 280 nm)	2.24	1.94	1.71	1.96
Anthocyanins	1.27	1.35	1.08	1.25
Total monomers	0.10	0.09	0.09	0.08
Catechin	0.04	0.04	0.04	0.03
Epicatechin	0.04	0.03	0.04	0.03
Epigallocatechin	0.01	0.02	0.02	0.01
Dimer B1	0.04	0.05	0.05	0.04
Dimer B2	0.03	0.03	0.03	0.03
Procyanidin polymers	0.65	0.55	0.55	0.51
Total polyphenols (HPLC)	0.98	0.88	0.89	0.82
1. Optical density				

There appears to have been some loss of procyanidin polymers, but not of anthocyanins, monomers, or dimers. Further testing of samples of the 2% aqueous solution of grape pomace extract was designed to assess the effect of variations in pH on solutions held at 6°C for either 1 or 7 days. The results of this third experiment are shown in Table 12.

Table 12. Stability of exGrape® Total Grape Pomace Extract at Different pH.

Parameter	Initial Value (%)	pH 1		pH 3.5		pH 7	
		24 Hours (%)	7 Days (%)	24 Hours (%)	7 Days (%)	24 Hours (%)	7 Days (%)
Total polyphenols (OD[1] at 280 nm)	2.24	1.79	1.79	1.87	1.81	1.85	1.78
Anthocyanins	1.27	1.25	1.14	1.21	1.10	0.63	0.63
Total monomers	0.10	0.07	0.07	0.07	0.09	0.05	0.06
Catechin	0.04	0.03	0.03	0.03	0.04	0.02	0.02
Epicatechin	0.04	0.03	0.02	0.03	0.03	0.02	0.02
Epigallocatechin	0.01	0.01	0.02	0.01	0.02	0.01	0.01
Dimer B1	0.04	0.04	0.05	0.04	0.05	0.02	0.04
Dimer B2	0.03	0.03	0.03	0.02	0.03	0.02	0.02
Procyanidin polymers	0.65	0.50	0.45	0.48	0.53	0.52	0.59
Total polyphenols (HPLC)	0.98	0.82	0.74	0.78	0.86	0.75	0.86
1. Optical density							

As was observed in the previous experiment, most of the loss of polyphenolic compounds was of procyanidin polymers, but some loss of anthocyanins and monomers was observed at neutral pH while these components remained more stable at acid pH.

These experiments show that exGrape® Total is stable even at 100°C for up to an hour and, particularly at acidic pH, at a moderately elevated temperature of 40°C for at least 3 months. It may be concluded that the product is stable under normal storage conditions.

3. INTENDED USE AND CONSUMER EXPOSURE

3.1. Intended Technical Effect

Grape pomace extract is intended for addition to certain categories of conventional foods as a source of antioxidants to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation (21 CFR §170.3[o]).

3.2. Intended Addition of exGrape® Total Grape Pomace Extract to Foods

The intended use of exGrape® Total grape pomace extract is identical to that of MegaNatural™ Gold grape seed extract (GSE) and grape skin extract (GSKE), which were the subjects of GRAS Notice No. 000125, submitted to FDA on February 21, 2003. The FDA response (in which GSKE was referred to as grape pomace extract [GPE]), dated August 18, 2003, stated that "the agency has no questions at this time regarding Polyphenolics' conclusion that GSE and GPE are GRAS under the intended conditions of use." These conditions of use include addition of GSE and GSKE/GPE, alone or in combination, to fruit juices for which no standard of identity exists, fruit-flavored beverages, fruit-flavored beverage mixes, and carbonated fruit-flavored beverages at a concentration of up to 210 parts per million.

It is intended that exGrape® Total grape pomace extract may be used as an alternative to other grape-derived extracts or in combination with them, with the total addition level of all grape-derived extracts combined not exceeding 210 parts per million.

Addition levels of grape pomace extract are limited by organoleptic considerations; the phenolic compounds can impart a bitter or astringent taste (Yoo et al. 2012). In one study in which grape seed extract was dissolved in white wine at a range of concentrations, Yoo et al. (2012) established "rejection thresholds" for Korean and Autralian consumers at 320 and 400 ppm, respectively.

It is important to recognize that although grape pomace extract is a deep purple color which, if added to foods at sufficiently high concentrations, would be capable of imparting significant color, the intended use levels do not reach concentrations at which any color imparted would be significant with regard to the appearance, value, marketability, or consumer acceptability of the product. Further, any slight color effect would be incidental to the use of grape pomace extract as an antioxidant and would not result from any intentional use for the purpose of imparting color.

3.3. Estimated Intakes of exGrape® Total Grape Pomace Extract

In GRN 000125, the 90th percentile per-user intake of GSKE and GSE was estimated to be about 130 mg/day based on data from the U.S. Department of Agriculture's 1994-96 Continuing Surveys of Food Intakes by Individuals and the 1998 Supplemental Children's Survey (GRN 000125, Table 6.1). In terms of body burden, the 90th percentile intake of GSKE and GSE was estimated at 4 mg/kg bw/day, based on the same survey data (GRN 000125, Table 6.2).

Since the intended use of exGrape® Total grape pomace extract is identical to that of the existing MegaNatural™ products, the estimated daily intake of exGrape® Total is the same as that reported in GRN 000125, i.e., 130 mg/person/day, equivalent to 4 mg/kg bw/day. Further, because exGrape® Total grape pomace extract merely provides an alternative to the food formulator, no additional consumer exposure to grape pomace extract will result from the use of the exGrape® Total brand[1]. Further, according to Table 3.3 in GRN 000125, the mean phenolic content (in gallic acid equivalents) of GSE is 95.17% and that of GSKE is 94.57%, while the mean total phenol content of exGrape® Total grape pomace extract is 73.42%. Thus, the consumer exposure to phenols from the intended use of exGrape® Total grape pomace extract will be lower than that expected from the same addition level of GSE and/or GSKE.

Earlier estimates of dietary intakes of polyphenols from naturally occurring sources were limited by the scarcity of analytic data and by inadequate and inconsistent analytic techniques, but this situation has been improving. In the United States, for example, the U.S. Department of Agriculture established a flavonoid database in 2003 with data for 225 foods; in release 2 in 2006, data were provided for 392 foods; and release 3 in 2011 included flavonoid content of 500 foods (USDA/ARS 2011). At an ILSI-sponsored workshop in 2005, the available literature on worldwide flavonoid sources and intake was assessed and provided in a published report (Erdman et al. 2005). Average intakes of polyphenols by adults in the United States and other countries were reported as summarized in Table 13.

Table 13. Estimated Consumption of Polyphenols by Adults (Erdman et al. 2005).

Polyphenol	U.S. Adults (mg/day)	Worldwide Adults (mg/day)
Flavonols	9.4	13.1 – 29
Flavones	1.3	1 – 2.1
Flavanones	2.7	20 – 78
Monomeric flavan-3-ols	4 – 121	11 – 50
Anthocyanidins	1.3	6.5
Proanthocyanidins	58	No estimate
Source: Erdman et al. 2005, Table 2.		

[1] A similar grape seed extract with less than 5.5% catechin monomers was the subject of GRN 000124; this product is likewise GRAS for use in fruit juices and fruit-flavored drinks, as well as in carbonated soft drinks, flavored-milk-based beverages, buttermilk, reduced-fat milks, milk-based meal replacements, yogurt, frozen yogurt, regular and low-fat ice creams and ice milks, instant and regular hot cereals, ready-to-eat cereals, mayonnaise, and health bars.

4. REVIEW OF SAFETY DATA

4.1. Kinetics and Metabolism

4.1.1. In Vitro Studies

Deprez et al. (2000) employed ^{14}C-labeled proanthocyanidins from willow leaves (*Salix caprea*) to study catabolism by human microbiota. The tested proanthocyanidins contained no dimers or trimers and had an average polymerization degree of 6, similar to that of the proanthocyanidins found in grape seeds. Aqueous solutions of labeled proanthocyanidins were added to fecal suspensions, which were then incubated in brain-heart medium with or without microbiota and samples were taken at 0, 6, 12, 24, 36, and 48 hours.

In the presence of human colonic microbiota, several metabolites were detected, accounting for 2.7% of the initial radioactivity after 48 hours. These metabolites were not detected in the absence of microbiota. The metabolites included 6 phenolic acids: 2-parahydroxyphenyl acetic acid and 2-parahydroxyphenyl propionic acid and their metahydroxy isomers, 5-metahydroxyphenyl valeric acid, and phenylpropionic acid. The authors concluded that, "These results show for the first time that proanthocyanidin polymers, which cannot be absorbed through the small intestinal barrier because of their high molecular weight, can be degraded by the colonic microflora into low-molecular-weight aromatic acids."

4.1.2. Animal Studies

Terrill et al. (1994) studied digestion of ^{14}C-labeled condensed tannins from *Lotus pedunculatus*, generally similar but not identical to proanthocyanidins in grape seed extract, in sheep. In one experiment using a single sheep—unidentified with regard to breed, sex, age, weight, or other characteristics—purified labeled condensed tannins were introduced through a cannula into the abomasum of a sheep held in a metabolism cage over a period of 400 minutes. The sheep was then euthanized and samples were taken of blood, digesta, and tissues. Of the administered radioactivity, 96.4% was recovered from the digesta and 3.5% from intestinal tissues. There was no detectable radioactivity in blood; trace radioactivity was detected in the liver, and 0.05% of the administered dose was recovered in urine and in feces. (Since no radioactivity was found it blood, it might be suggested that the radioactivity found in urine resulted from fecal contamination.) The authors interpreted the results as suggesting "that little if any of the ^{14}C-labeled condensed-tannin-carbon was absorbed from the small intestine."

Tsang et al. (2005) investigated the absorption, metabolism, and excretion of flavan-3-ols and procyanidins following gavage administration of grape seed extract by male Sprague-Dawley rats (age not reported) weighing 250±5 g. Grape seed extract was produced by Paroeno (Bordeaux, France) from Chardonnay and Pinot Noir grape seeds using water and alcohol extraction. Analysis of the phenolic content showed about 30% (+)-catechin, 19% (-)-epicatechin, 15% procyanidin-O-gallate, 8% procyanidin dimer B_1, 8% procyanidin dimer B_2, 4% procyanidin dimer B_4, 4% procyanidin trimer, 4% (-)-epicatechin-3-O-gallate, and lower levels of other polyphenolics. Twenty-four rats weighing 250±5 g were individually housed in metabolic cages and given single gavage doses of 1000 mg grape seed extract/kg bw. At 0, 1, 2, 3, 4, 6, 12, and 24 hours post-dosing, 3 animals were terminally anesthetized; blood was removed by cardiac puncture, urine and feces were collected, and liver, kidney, brain, stomach,

duodenum/jejunum, ileum, cecum, and colon were excised. All samples were analyzed for phenolic content.

Phenolic compounds isolated from the gastrointestinal tract—stomach, duodenum and jejunum, ileum, cecum, and colon—were intact flavan-3-ols as found in the grape seed extract with only trace evidence of catabolism and no indication of depolymerization of proanthocyanidins. The only phenols detected in plasma were catechin glucuronides and methylated glucuronide metabolites; these compounds were also found in liver and kidneys, as well as in urine, which also contained trace amounts of procyanidin dimers and trimer C_2. Over 24 hours, 27% of ingested (+)-catechin and 36% of (-)-epicatechin metabolites were excreted in the urine. The authors noted that, while these percentages are higher than have been previously obtained, they still leave about 70% of the ingested monomer unaccounted for—neither being absorbed nor reaching the colon—and suggested that "the most likely fate of these compounds is that they are converted to low-molecular weight phenolic acids" such as 3-hydroxyphenylpropionic acid.

The bioavailability of gallic acid and catechins from grape seed extract was investigated in two experiments with a total of 32 male Sprague-Dawley rats weighing 275-300 g (Ferruzzi et al. 2009). The test article, MegaNatural-AZ® Grape Seed Polyphenolic Extract, contained 16.4% (-)-epicatechin, 14.7% (+)-catechin, and 9.1% gallic acid, as well as proanthocyanidin dimers and trimers. In the first experiment, rats (number not reported) were implanted with jugular catheters; after recovery from surgery they received acute doses of 50, 100, or 150 mg grape seed extract/kg bw by intragastric gavage, providing the equivalent of doses of 483, 967, and 1451 mg in a 60-kg human. Blood was collected via the jugular catheter at 0, 0.5, 1, 2, 4, 6, and 8 hours after gavage and brains were excised at 8 hours post-gavage.

The effect of repeated exposure was assessed in the second experiment, an escalating-dose study in which rats (number not reported) received 50 mg/kg bw on days 1 and 2, 100 mg/kg bw on days 3 and 4, and 150 mg/kg bw on days 5-10. Blood was collected via the jugular catheter at 0, 0.5, 1, 2, 4, 6, and 8 hours after the final gavage. Brains were also excised 8 hours post-gavage.

No deaths were reported in either experiment, nor did the authors report any effect on bodyweight or other indicators of well-being. Catechin and epicatechin monomers and gallic acid were detected in rat plasma following acute oral administration of grape seed extract, but dimers, trimers, and oligomers were not detected. Methylated metabolites including 4-O-methylgallic acid, 3'-O-methylcatechin, and 3'-O-methylepicatechin were also observed in plasma, indicating a degree of metabolism. At the lowest acute dose, methylated derivatives represented about 8-10% of total catechin and epicatechin, while at the highest acute dose level the methylated metabolites accounted for 23-28% of the total; repeated dosing did not increase the proportion of methylated derivatives. Methylated metabolites of gallic acid, on the other hand, increased significantly with repeated dosing over the levels observed with acute administration of grape seed extract, from between 15 and 18% to about 28% of total gallic acid.

Catechin, epicatechin, and gallic acid reached peak levels 1-2 hours after gavage, returning to baseline by 8 hours. Increasing the acute dose of grape seed extract from 50 to 150 mg/kg bw approximately tripled the C_{max} for these monomers, but did not significantly increase plasma levels of gallic acid. Repeated administration of grape seed extract significantly increased blood levels of all measured phenols.

No free phenolics or their methylated derivatives were found in brain tissue following acute administration at any tested dose, but trace levels of catechin, epicatechin, and gallic acid were found after 10-day repeated dosing. The authors concluded that "absorption and brain accumulation of small molecular weight grape seed extract constituents, namely gallic acid and catechins, is enhanced by repeated dosing... more complex polyphenolic compounds [dimers, trimers, and oligomers] are believed to be fermented by intestinal microflora leading to the production of smaller molecular weight phenolic acids."

The pharmacokinetics and tissue distribution of grape polyphenols were assessed in Sprague-Dawley rats using ^{14}C-labeled polyphenols (Janle et al. 2010). Cell suspension cultures of hybrid grapes were prepared with ^{14}C-sucrose as a carbon source during fermentation. The cultures were harvested, extracted, and fractionated into 5 test articles with ^{14}C-enrichment levels ranging from 5% to 69%. One fraction consisted primarily of monomeric proanthocyanidins, a second of proanthocyanidin monomers through hexamers, a third of proanthocyanidin dimers and trimers and anthocyanin glycosides, a fourth of anthocyanin 3-O-glycosides, and a fifth of anthocyanin glycosides. Thirteen 59-66-day-old male Sprague-Dawley rats weighing ~250 g were implanted with jugular catheters and dosed with 0.5 ml of one of the fractions (2 rats/ fraction with a third rat receiving fraction 3 and 2 additional rats receiving fraction 4). Blood was sampled over 24 hours and subcutaneous interstitial fluid was sampled hourly; urine and feces were collected. After 24 hours the rats were sacrificed, the vascular system was flushed with saline, and the brain was removed.

Fraction 5 reached peak blood concentration in 15 minutes while fraction 3 required 4 hours; the other 3 fractions peaked at about 30 minutes. Fraction 5 also had the highest rate of absorption—particularly of its anthocyanin glycosides— with an area under the curve representing 3.5% of the dose, and the fastest rate of elimination with a half-life of about 3 hours. Maximum concentrations in interstitial fluid occurred later than in blood with an average of 2-3 hours. Over 40% of the doses of fractions 3 and 5 were eliminated in the urine and less than 5% in feces; on the other hand, only 3% of fraction 2 was excreted in urine while 19% was found in feces.

With regard to the capability of different fractions to reach the brain and cross the blood-brain barrier, the authors concluded: "... small-molecular-sized, polar polyphenols, such as anthocyanin 3-O-glysides and cyanidin glycosides, as well as anthocyanin glucosides such as peonidins and cyanidins or metabolites of these, accumulate in brain tissue. In contrast, dietary proanthocyanidins and their metabolites were found in very low levels in brain tissue."

Rzeppa et al. (2012) studied metabolism and urinary excretion of procyanidins in the absence of flavan-3-ols in pigs, noting that "a transfer of these results to humans is possible due to their similar gastrointestinal tract." Procyanidins A2, B1, B2, B3, B4, B5, B6, B7, B8, C1, and C2 were isolated from plant sources or synthesized and added to the feed of 3 castrated male crossbred German Landrace x Pietrain pigs weighing a mean of 46.6 kg, housed in individual metabolic-balance cages, to provide a daily dose of 250 mg/kg bw; 2 similar pigs served as controls. Urine was collected at 0, 3, 6, 9, 12, 24, 27, and 30 hours.

Total urinary excretion of procyanidins ranged from 0.004% of the administered C1 trimer to 0.019% of the administered B4 dimer, with the maximal concentrations occurring at 6 hours. No methyl derivatives or glucuronide adducts of procyanidins could be identified, while flavan-3-ols were metabolized to a greater extent by methylation and glucuronidation with over

90% of the analytes in the form of methyl derivatives or conjugated with glucuronic acid. The authors concluded that "absorption of dimeric and trimeric procyanidins is probably poor considering the total excretion as percentage of total intake." They continued, "In comparison to procyanidins, flavan-3-ols, catechin, and epicatechin were excreted in distinct higher concentrations that indicate their higher bioavailability."

4.1.3. Human Studies

In a randomized unblinded crossover study (Caccetta et al. 2000), 12 apparently healthy males aged 40-63 years consumed 5 ml/kg bw of red wine, red wine with the phenolic content removed, non-alcoholic red wine, or water over a period of 30 minutes. Tests with each beverage were in random order and separated by at least 1 week. Blood samples were taken at 0, 1, 2, and 4 hours from the beginning of the intake period and analyzed for caffeic, protocatechuic, and 4-*O*-methylgallic acids as well as total polyphenols, ethanol, uric acid, and LDL oxidizability.

Serum ethanol concentrations increased significantly from 0.01% to a maximum of 0.06% after consumption of the alcohol-containing beverages, and caffeic and 4-*O*-methylgallic acids increased significantly after ingestion of the red wine and non-alcoholic red wine, but levels of protocatechuic acid and total polyphenols did not change. (Caffeic acid increased from abut 35 to 80-90 nmol/L after 1-2 hours while 4-*O*-methylgallic acid increased from trace levels to 170-180 nmol/L after 2-4 hours and levels of protocatechuic acid remained at about 100 nmol/L.) Phenol levels were not affected by the presence of ethanol. Uric acid concentrations increased significantly from 0.35-0.36 to 0.39-0.40 mmol/L in response to ingestion of all beverages except water. However, none of the beverages affected LDL oxidation.

Four apparently healthy adults, not further described, consumed capsules providing 2000 mg grape seed extract and had blood drawn 0 and 2 hours after intake (Sano et al. 2003). The test article was composed of 89% proanthocyanidins (0.9 % procyanidin B1) and 6% monomers. The dose was set at 2000 mg because a pretest with 400 mg resulted in no detectable proanthocyanidins in blood. With the dose of 2000 mg, traces of procyanidin B1 were detected in serum with a concentration of 10.6±2.5 nmol/L. This concentration is equivalent to about 5.78 µg/L, or a total blood content of about 27.2 µg procyanidin B1. Since the administered dose was 18 mg procyanidin B1 (0.9% of 2000 mg), the absorption was only about 0.15%. Although no HPLC traces were seen corresponding with other proanthocyanidins, the authors argued that the absorption of the relatively low-molecular-weight procyanidin B1 "shows the possibility that other proanthocyanidins can also be absorbed."

The metabolic fate of grape polyphenols was studied in 58 adult men and women who participated in a randomized, double-blind, placebo-controlled, double-crossover trial (van Dorsten et al. 2010). The study population included 33 men and 25 women aged 18-70 years with mild hypertension. In the first phase, 29 volunteers received ingested capsules with either a mixed extract of red wine and red grape juice or microcrystalline cellulose placebo for 4 weeks, followed by 4 weeks crossover with no washout period. In the second phase, 29 different volunteers followed a similar design but with an extract of red grape juice only rather than a mixed extract. At the end of each test period, 24-hour urine and fasted blood samples were collected.

Both of the tested extracts consisted of >80% polyphenols (primarily oligomeric proanthocyanidins) and provided 800 mg/day of gallic-acid equivalents. The primary differences

between the two extracts were that the mix had about 3% catechins and procyanidin dimers and trimers while the juice extract had none, and the juice extract contained 23% anthocyanins compared to only about 9% in the mixed extract.

Ingestion of the mixed extract resulted in a significant increase in the hippuric acid levels of the urine over placebo or red-grape-juice extract (+33%, from 0.99 to 1.32 g/day) and a lesser but still statistically significant increase in low-weight phenolic acids (with increases in individual acids ranging from -6% to +113%). The authors concluded that the metabolomics approach of this study was able to detect marginally different impacts on urine metabolic profiles following consumption of high-polyphenol extracts differing only slightly in composition, and that "4-week consumption of polyphenol-rich wine and grape supplements results in the elevated excretion of a wide range of phenolic acids, which is in line with extensive gut microbial metabolism of grape/wine polyphenols."

Manach et al. (2005) reviewed 97 studies that investigated the kinetics of polyphenol absorption among adults based on ingestion of single doses. They concentrated on individual polyphenols and their metabolites and attempted to determine bioavailability, time and level of maximum plasma concentration, elimination half-life, and relative urinary excretion. Absorption of anthocyanins appeared to be extremely low, with doses of up to one gram resulting in maximal plasma concentrations of only 10-15 nmol/L and urinary excretion of 0.004% to 0.1% of intake. However, the authors suggested that this may underestimate bioavailability: the only metabolites of anthocyanins detected were unchanged glycosides, but it is possible that glucuronidated and sulfated derivatives, which are unstable, may have degraded in samples prior to analysis. The anthocyanins are rapidly absorbed with an average time to C_{max} of 1.5 hours for plasma and 2.5 hours for urine.

In the 24 studies reviewed by Manach et al. (2005) that assessed the bioavailability of flavonols, absorption ranged from 0.07% to 6.4%. The kinetics of flavonols were generally less rapid than anthocyanins, with a slightly longer time to plasma C_{max} and a much longer excretion half-life of 11 to 28 hours.

The bioavailability of different catechins differed markedly in the 28 studies reviewed by Manach et al. (2005) from only trace absorption to over 50%, although absorption may have been underestimated because most studies did not analyze methylated metabolites. The mean time to plasma C_{max} was about 2 hours and elimination was rapid with a time to urinary C_{max} not much longer. Most catechins are excreted in urine, but galloylated catechins are eliminated in the bile. Manach et al. (2005) found no evidence of any absorption of polymeric proanthocyanidins as such and only trace absorption of dimers. However, there is some evidence of absorption of aromatic acids resulting from microbial fermentation of proanthocyanidins.

Manach et al. (2005) reviewed 12 studies that included assessment of hydroxycinnamic acids, finding absorption varying greatly for the various hydroxycinnamic acids, from only trace levels of chlorogenic acid to over 50% of ferulic acid. Absorption was relatively rapid with a mean plasma T_{max} of about an hour; no data were available regarding elimination rate. In five studies assessing bioavailability of gallic acid, it appeared to be well absorbed compared with other polyphenols; plasma concentrations reached 4 µmol/L after ingestion of 50 mg gallic acid and over 36% of ingested gallic acid was recovered in urine. The elimination half-life was 1.1-1.5 hours.

Manach et al. (2005) concluded that, "The polyphenols that are most well absorbed in humans are isoflavones and gallic acid, followed by catechins, flavanones, and quercetin glucosides, with different kinetics. The least well-absorbed polyphenols are the proanthocyanidins, the galloylated tea catechins, and the anthocyanins."

4.1.4. Conclusions Regarding Pharmacokinetics

Pharmacokinetic studies have been conducted with a variety of extracts, some commerical products and some specially formulated in the laboratory. While the specific extract that is the subject of this GRAS determination, exGrape® Total, has not been subjected to pharmacokinetic study, all of its polyphenolic constituents are found in the tested formulations and thus have been investigated. It is reasonable to conclude that the results of this research are fully valid for exGrape® Total.

Kidd (2009) observed that "preparations of [grape seed polyphenols] vary greatly in chemical profiles, making study interpretation challenging. One important aspect is that the higher molecular weight polyphenols tend to be very poorly bioavailable." Animal and human studies have demonstrated that the bioavailability of grape seed extract phenolics, including gallic acid and proanthocyanidin monomers such as catechin and epicatechin, is low (< 2% of the ingested dose) and that of proanthocyanidin dimers, timers, and oligomers even lower. These more complex polyphenolic compounds are not absorbed intact to a significant degree, but are fermented by intestinal microorganisms, producing smaller molecular weight phenolic acids, which may be more bioavailable than the parent compounds. Manach et al. (2005) estimated that microbial metabolites such as 5-(3',4'-, or 5-(3',5'-, or 5-(3',4',5'- trihydroxyphenyl) valerolactone accounted for 8-25 times the plasma and urine levels measured for the unchanged compounds. Similarly, proanthocyanidins are fermented into aromatic acids, including metahydroxyphenylpropionic acid, metahydroxyphenylacetic acid, and their parahydroxy isomers, which are readily detected in plasma and urine after ingestion of proanthocyanidins. Intestinal absorption of polyphenolics is due to active transporters, including the monocarboxylic acid transporter (Vaidyanathan and Walle 2001). After absorption, phenolic compounds may be metabolized (methylated derivatives of gallic acid and proanthocyanidin monomers have been isolated from plasma) and secreted into circulation, or effluxed back to the intestinal lumen in bile.

Elimination of the relatively well-absorbed phenolic monomers is rapid, with half-lives of 1.1 to 1.5 hours. Manach et al. (2005) concluded that these compounds "have no chance to accumulate in plasma with repeated ingestion," although they also noted the extensive variability—10-fold or greater variations—observed in pharmacokinetic studies.

4.2. Toxicological Studies

A wide variety of extracts derived from grapes, grape seeds, and grape skins has been subjected to toxicological investigation, as well as many individual phenolic compounds. While the various extracts differ in the distribution of the polyphenols found in them, all of them are generally similar with regard to the overall array of polyphenols present and, the findings of toxicity testing are generally applicable to all of these extracts. This similarity and validity of inferences to related extracts was observed by the National Toxicology Program (NTP) when it selected a single extract for testing as "representative of dietary supplements with activity based

on [oligomeric proanthocyanidins]" (NTP 2000). Toxicity studies are summarized in Table 14at the end of the section.

4.2.1. Acute Oral Toxicity

Ray et al. (2001) gavaged rats with 5000 mg grape seed extract/kg bw to determine its acute toxicity. The test article was ActiVin®, a water-ethanol proanthocyanidin extract from grape seeds, shown by HPLC and GC/MS methods to contain 74% oligomeric proanthocyanidins (54% dimers, 13% trimers, and 7% tetramers) as well as "a small amount" of catechin derivatives and other flavonoids. Five "young adult" albino rats of each sex weighing 225-280 g (strain, age, and mean body weight were not reported) were acclimated for 7 days with Purina Certified Rodent Chow and tap water available *ad libitum* prior to administration of the test material. The rats were observed at 1, 3, and 4 hours post-dosing and twice daily for 14 days; body weights were measured on days -1, 0, 7, and 14. After sacrifice, the stomach, adrenal glands, brain, intestine, esophagus, eyes, heart, kidneys, liver, mesenteric lymph node, lungs, mammary gland, ovaries, pancreas, pituitary gland, salivary gland, skin, spleen, thymus gland, thyroid gland, trachea, urinary bladder, and uterus were excised and examined.

One female died on day 1; red matting adhered to the tail and brown material to the nonglandular portion of the stomach, but no other significant changes were observed in any tissues at necropsy. Seven animals had wet or dried brown or yellow urogenital staining and mucoid feces. All living animals appeared normal by day 3 and there were no other deaths and no significant effects on body weight or examined tissues. The authors reported that "the LD_{50} of [grape seed extract] was found to be greater than 5000 mg/kg when administered once orally to fasted male and female albino rats."

Yamakoshi et al. (2002) tested the acute oral toxicity of grape seed extract in 4-week-old male and female Fischer 344/DuCrj rats (body weights not reported). The extract, prepared in the laboratory, was derived from grape seeds by water-ethanol extraction, condensed, filtered, and spray-dried. Based on HPLC, the content of the extract was estimated as 89.3% proanthocyanidins (6.6% dimers, 5.0% trimers, 2.9% tetramers, and 74.8% oligomers and polymers), 6.6% monomeric flavanols [2.5% (+)-catechin, 2.2% (-)-epicatechin 1.4% (-)-epigallocatechin, and 0.5% (-)-epigallocatechin gallate], 2.24% moisture, 1.06% protein, and 0.8% ash. The rats were divided into 3 groups with 5 rats/sex/group, given free access to feed and water, and given grape seed extract by oral gavage at doses of 0, 2000, or 4000 mg/kg bw. The rats were observed for 14 days, after which the animals were sacrificed and necropsied.

There were no deaths or clinical signs; no abnormalities were seen at necropsy. The LD_{50} for oral toxicity of the tested grape seed extract in male and female Fischer 344 rats in this study was > 4000 mg/kg bw.

El-Adawi et al. (2006) extracted dried ground grape seeds with aqueous ethanol for 120 minutes at 70°C, resulting in an extract containing 598.8 mg polyphenols/g. Thirty-six male Wistar rats aged 35 days and weighing 100-120 g were assigned to 6 groups (n = 6 rats/group) to receive by oral gavage single doses of 0, 3000, 4630, 6380, 9000, or 12650 mg grape seed extract/kg bw, providing 0, 1796, 2773, 3820, 5394, or 7575 mg polyphenol/kg bw, respectively. The rats were observed for 14 days. There were no deaths or other adverse effects among the control rats or those in the two lowest dose groups, and 1, 3, and 4 deaths in the mid-dose, mid-high-dose, and high-dose groups, respectively. The rats in these 3 groups also exhibited

significantly reduced feed consumption, but no other clinical signs preceding death. The LD_{50} was calculated using a probit analysis to be 6300 mg grape seed extract/kg bw among male Wistar rats.

In a study only marginally related to the assessment of the oral toxicity of grape seed extract, but included for completeness since the test article, procyanidin dimer B2, is found at a concentration of about 11 mg/g in exGrape® Total, Takahashi et al. (1999) evaluated the acute subcutaneous toxicity of procyanidin dimer B2 by administration of doses of 0, 500, 1000, or 2000 mg/kg bw to groups of 5 male and 5 female 6-week-old Sprague-Dawley rats weighing 204-226 g (males) and 151-175 g (females). The rats were observed for 14 days, then sacrificed and necropsied. Neither housing nor feed intake were reported. None of the animals died. A decrease in general activity and body weight was noted in the highest-dose rats between 6 and 24 hours, but all animals recovered by 48 hours. At necropsy, subcutaneous granulomatous inflammation and accumulations of pigment-laden macrophages in the duodenal mucosa were observed in the higher-dose-group animals of both sexes. The authors did not regard these effects as toxicologically significant and concluded that the lethal subcutaneous dose of procyanidin dimer B2 is > 2000 mg/kg bw.

4.2.2. Subchronic Oral Toxicity

4.2.2.1. Rats

The repeated-dose oral toxicity of 2 grape extracts was studied by Bentivegna and Whitney (2002) using male and female Sprague-Dawley Crl:CD® IGS BR rats in a Redbook-compliant 90-day feeding study. The test articles were MegaNatural™ grape seed extract (containing 90.5% [w/w] total phenols, 4.8% catechin, 4.4% epicatechin, and 1% gallic acid, with no detectable anthocyanins; the extract included 10.4% monomers, 74.9% oligomers, and 14.7% polymers) and MegaNatural™ grape skin extract (containing 87.3% [w/w] total phenols, 3.4% catechin, 4.6% epicatechin, 2.4% gallic acid, and 2.6% total anthocyanins; including 16.7% monomers, 67.4% oligomers, and 15.9% polymers). After 2 weeks acclimatization the rats were 6 weeks old; males weighed 188-237 g (mean = 211 g) and females weighed 128-172 g (mean = 149 g). Groups of 20 rats/sex/dose were individually housed and fed diets of Certified Rodent Diet No. 502 containing no grape extract; 2.5% grape skin extract; or 0.625, 1.25, or 2.5% grape seed extract for 90 days. Chow and water were available *ad libitum*. Indirect ophthalmologic examinations were performed pretest and at termination of dosing. Animals were observed in their cages twice a day for mortality and clinical signs and were removed from the cage weekly for weighing and more detailed observation of skin and fur, eyes, nose, oral cavity, external genitalia, autonomic activity, changes in gait or posture, and response to handling. Feed intake was measured weekly.

Orbital sinus blood samples were taken from 10 animals/sex/group at one month and aortic blood samples were taken from the remaining rats at termination. Hematological measures included hemoglobin concentration, hematocrit, erythrocyte count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total leukocyte count, reticulocyte count, and differential leukocyte count. Coagulation measures were prothrombin time and activated partial thromboplastin time, and clinical chemistries included AST, ALT, ALP, BUN, creatinine, glucose, TC, TG, total protein, albumin, total bilirubin, sodium, potassium, chloride, calcium, inorganic phosphorus, and γ-glutamyl transferase. After sacrifice, adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary

gland, prostate gland, spleen, testes, thymus, thyroid/parathyroid, and uterus with cervix were excised and weighed. Macroscopic histopathological examinations were performed on adrenal glands, aorta, bone and marrow (sternum and femur), brain (medulla, pons, cerebrum and cerebellum), esophagus, heart, kidneys, lacrimal glands/Harderian glands, large intestine (cecum, colon, rectum), larynx, liver, lungs (with main-stem bronchi), lymph nodes (mesenteric, mediastinal), mammary gland, nerve (sciatic), ovaries, pancreas, pharynx, pituitary gland, prostate gland, salivary gland (submandibular), seminal vesicles, skeletal muscle, skin, small intestine (duodenum, ileum, jejunum), spinal cord (cervical, thoracic, lumbar) spleen, stomach, thymus, thyroid/parathyroid glands, trachea, urinary bladder, uterus with cervix, vagina, Zymbal's gland and tissues with lesions or masses. Tissues from the control group and the two groups receiving feed with 2.5% extract were examined microscopically.

Analyses of samples of feed confirmed the stability, homogeneity, and concentrations of the admixtures of the test articles. There was no mortality and physical observations were unremarkable; no ophthalmologic findings were regarded as test-article related. Male rats, but not female rats, receiving feed containing 2.5% grape seed or grape skin extract consumed significantly more feed throughout the study (suggested by the authors as compensation for the lower caloric value of the feed); mean body weights and body weight gain were similar in all groups. No significant test-article related changes in hematology, coagulation parameters, or clinical chemistries were noted at 1 month or at termination in either males or females. The only statistically significant difference in organ weights was decreased absolute and relative heart weights in female rats receiving grape skin extract at 2.5% dietary concentration; this difference was not observed in males, did not exhibit dose-response, and was not accompanied by microscopic pathology and was consequently regarded as not related to treatment. No histopathology was observed in males or females receiving 2.5% grape seed extract. Male rats receiving 2.5% grape skin extract exhibited a significant increase in the occurrence of renal cortical inflammation comprising predominantly lymphocytic interstitial infiltrates. The inflammation was not severe and was reported by the authors to be common in aging male rats; it was not regarded as treatment related. The NOAELs were considered to be 2.5%, the highest concentration levels tested of both extracts, and the authors concluded:

> "Results of the current study strongly support the safety of GSE [grape seed extract] and GSKE [grape skin extract] as dietary components for human consumption. The no-observed-adverse-effect level (NOAEL) was considered to be approximately 2150 mg/kg bw/day for administration of GSE as well as GSKE to female rats while approximately 1780 mg/kg bw/day was considered a NOAEL in male rats. These values represent the time-weighted mean dose rates occurring in the high-dose groups over the course of the study."

Wren et al. (2002) evaluated the subchronic oral toxicity of a grape seed extract designated IH636, an ultrafiltered water extract containing 76.3% oligomeric polyphenols and 2.8% monomeric proanthocyanidins. The study was reported to be compliant with FDA's GLP regulations but no statement was made regarding compliance with OECD or Redbook guidelines. Four groups of 9-10-week-old male and female Sprague-Dawley rats weighing 216-280 g (males) and 175-215 g (females) received diets of Purina Certified Rodent Chow 5002 containing admixtures of IH636 of 0, 0.5, 1.0, or 2.0% (n = 20 rats/sex/group) for 90 days. Animals were housed 2-3 rats/cage with chow and water available *ad libitum*. The animals were observed 1-2 times/day and removed from the cage for more detailed clinical observations and

weighing daily on days 1-15 and weekly thereafter. Feed consumption was recorded twice weekly. Ophthalmologic examinations were performed prior to study initiation and during the final week. Blood samples were drawn from the retro-orbital sinus on days 91-94 for analyses of hematological parameters (red blood cell count, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, white blood cell count and differential counts [including absolute banded neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils], platelet count, and reticulocyte count) and clinical chemistries (AST, ALT, ALP, albumin, total bilirubin, BUN, calcium, chloride, TC, creatinine, globulin, glucose, phosphorus, potassium, total protein, sodium, iron, total iron binding capacity, and iron/total iron binding capacity). After the blood draws the animals were euthanized and subjected to gross necropsy, including examination of all body surfaces and orifices and all cranial, thoracic, and abdominal organs. The following tissues were sectioned for histopathological examination: adrenal glands, aorta, urinary bladder, bone and marrow (from sternum), brain, cecum, cervix, colon, duodenum, esophagus, epididymides, eyes, femur, gross lesions (including tissue mass and abnormal regional lymph nodes, if identified), heart, ileum (including Peyer's patches), jejunum, kidneys, lungs and bronchi, liver, lymph nodes (mesenteric), mammary gland (to include nipple and surrounding tissue), ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, skeletal muscle, skin (abdominal; taken with mammary gland), spinal cord (mid-thoracic), spleen, stomach, testes, thymus, thyroid and parathyroids, tongue, trachea, uterus, and vagina. Organ weights were recorded for all animals for the following (paired organs were weighed together): prostate gland and seminal vesicles, adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, testes, uterus, and thymus.

Analyses of the chow confirmed the concentrations, content uniformity, and stability of the grape seed extract. There were no unscheduled deaths. Some hair loss was observed in small numbers of male and female rats in all groups, not thought to be compound-related. There were no significant clinical observations and no differences or changes were noted during the ophthalmoscopic examinations. Significantly increased consumption of feed was observed in both male and female rats but was more consistently statistically significant in the males. The authors suggested that the rats may have preferred the flavor and texture of the chow containing grape seed extract, although it may be suggested that the lower energy content of the test diets may have contributed. Slightly reduced weight gain was noted in female rats in the 1.0 and 2.0% extract groups, but was statistically significant only in the subgroup necropsied on day 91 and not in the subgroups necropsied on days 92-94. The authors stated that, "Because the difference in body weights was only slight, and given the fact that the effect was was statistically significant only in the day-91 necropsy subgroup, this effects was considered to be of no biological significance." No adverse effects were seen during necropsy, in comparison of absolute and relative organ weights between groups, or in histopathology. There were few significant differences between groups in hematological and biochemical values: male rats in the 1.0 and 2.0% groups exhibited increased serum sodium and decreased serum iron, while females in the 2.0% group had reduced BUN and creatine levels, but these changes were not accompanied by histopathological findings, were within normal historic ranges, and were regarded as toxicologically insignificant.

The authors noted that ingestion of grape seed extract at dietary levels of up to 2.0%, providing intakes of 1586 mg/kg bw/day for males and 1928 mg/kg bw/day for females, was

well tolerated by male and female Sprague-Dawley rats. They concluded that "at 2.0% in the diet, [grape seed extract] produced no significant compound-related toxicity in Sprague-Dawley rats." While the authors did not state their results in terms of a NOAEL, it is apparent that the NOAEL in this study was the highest concentration tested, equivalent to 1586 mg grape seed extract/kg bw/day for males and 1928 mg/kg bw/day for females.

Yamakoshi et al. (2002) tested the subchronic oral toxicity of grape seed extract in 5-week-old male and female Fischer 344/DuCrj rats (body weights not reported). The extract, prepared in the laboratory, was derived from grape seeds by water-ethanol extraction, condensed, filtered, and spray-dried. Based on HPLC, the content of the extract was estimated as 89.3% proanthocyanidins (6.6% dimers, 5.0% trimers, 2.9% tetramers, and 74.8% oligomers and polymers), 6.6% monomeric flavanols [2.5% (+)-catechin, 2.2% (-)-epicatechin 1.4% (-)-epigallocatechin, and 0.5% (-)-epigallocatechin gallate], 2.24% moisture, 1.06% protein, and 0.8% ash. The test article was administered for 90 days as an admixture in standard CRF-1 chow at concentrations of 0, 0.02%, 0.2%, and 2% with 10 animals/sex/group. Caging arrangements were not described; the animals had *ad libitum* access to feed and water. The rats were observed daily and feed consumption, water intake, and body weight were recorded weekly. After sacrifice, fresh urine was collected and analyzed for protein, sugar, ketone bodies, pH, and occult blood; blood was collected and evaluated for hematology (counts of red and white blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, platelets, mean corpuscular hemoglobin concentration, reticulocytes, and differential leukocytes) and clinical chemistry (total protein, BUN, AST, ALT, ALT, urea, albumin, bilirubin, creatinine, glucose, TC, TG, lactate dehydrogenase, Na, K, Ca, Cl, and P). Brain, pituitary, thyroid with parathyroid, thymus, lung, heart, liver, spleen, kidneys, adrenals, stomach, testes, ovaries, epididymides, seminal vesicles, prostate, uterus, submandibular glands, and urinary bladder were excised and weighed. In the high-dose and the control animals, all of the organs listed above plus the thoracic aorta, trachea, tongue, esophagus, duodenum, ileum, jejunum, cecum, colon, rectum, pancreas, lymph nodes (mandibular and mesenteric), mammary glands, spinal cord, sciatic nerves, skin, eyes, optic nerves, Harderian glands, sternum, femur, and skeletal muscles were examined histopathologically. All listed tissues were grossly examined in all animals.

There were no deaths and no abnormal clinical signs, nor significant differences in feed or water consumption or body weight. No significant differences were seen in urinanalytical, hematological, or biochemical measures. The mid-dose group of male rats had significantly higher absolute epididymides and lower absolute thymus weights than the controls while the female mid-dose group had significantly lower relative thymus weights and the female low-dose group had significantly lower relative stomach weights than the controls. These observations did not show dose-dependence and were not accompanied by histopathology, and so they were not regarded as evidence of toxicity. Neither gross examination nor histopathology revealed any treatment-related changes.

Based on feed consumption, the calculated intake of grape seed extract among male rats was 13.3, 129.1, and 1409.8 mg/kg bw/day in the low-, mid-, and high-dose groups, respectively. Among females, the grape seed extract intakes of the 3 groups were, respectively, 14.8, 154.0, and 1501.1 mg/kg bw/day. Thus, the NOAEL of grape seed extract in Fischer rats was 2% dietary concentration, the highest level tested, corresponding to 1410 mg/kg bw/day in males and 1501 mg/kg bw/day in females.

4.2.2.2. Dogs

Becci et al. (1983a) administered Welch's Special Grape Color Powder Type BW-AT to male and female beagle dogs as dietary admixtures of 0, 7.5, and 15% (w/w) for 90 days; no statement was made regarding compliance with OECD or Redbook guidelines. The test article consisted of a powder comprising 40% (w/w) grape extract in a maltodextrin carrier. No information was provided regarding the source, production method, or phenolic content of the grape extract, although the introduction in Becci et al. (1983b), discussed in the next section, implies that it was derived from fermented grape skin by acidic aqueous extraction and that the pigments present were anthocyanins. Groups of 4 pure-bred beagle dogs of each sex, weighing 6-10 kg at the beginning of the study (age and mean weight were not reported) were fed diets containing 0, 7.5, or 15% grape color powder or a second control diet containing 9% (w/w) maltodextrin. The animals were individually housed in wire-mesh-bottomed cages with free access to tap water and fed Purina Dog Chow for 1 hour/day.

The dogs were observed daily for clinical signs; feed consumption and body weight were measured weekly. After 7 and 13 weeks blood was collected for analysis of hematocrit, total and differential leukocyte counts, sedimentation rate, platelet count, reticulocyte count, hemoglobin, proteins, glucose, BUN, bilirubin, sodium, potassium, chloride, AST, ALT, and ALP, and urine was analyzed for pH, specific gravity, color, appearance, ketones, proteins, bilirubin, occult blood, and sediment. Ophthalmological examinations were given at the beginning and end of the study. At the end of the study, the animals were sacrificed and given a complete gross necropsy, including weighing of the brain, pituitary gland, heart, thyroid gland, liver, kidneys, spleen, adrenal glands, and testes or ovaries. Macroscopic histopathology was performed on the organs weighed and the pancreas, lung, epididymides and prostate or uterus, skeletal muscle, sciatic nerves, gall bladder, stomach, small intestine, large intestine, urinary bladder, lymph node, salivary gland, mammary gland, spinal cord, sternum and marrow, eyes, skin, and all gross lesions.

Behavior and physical appearance were unremarkable, although the feces of animals fed grape color powder were purple in color. There were no deaths and ophthalmic examination revealed no treatment-related effects. Feed consumption was unaffected, but the bodyweight gain of dogs of both sexes at the highest extract concentration was significantly reduced compared to controls. No differences were noted in any measures of hematology or clinical chemistry or in the urinalysis, there were no differences in absolute or relative organ weights, and no indication of any toxic or pathological effect was revealed in the gross necropsy or histopathological examinations. The authors attributed the decreased weight gain in dogs receiving diets with 15% grape extract to the decreased energy content of the diet, and concluded that "No evidence of toxic effect or pathological change was observed in male and female beagle dogs fed grape colour powder for 13 wk at doses of up to 15% of the diet."

The authors did not report the animals' feed intake, but based on data provided by Gad (2006), the dogs' average food intake was likely about 300 g/day by the males and 280 g/day by the females, and the intake of grape color powder by animals in the highest dietary concentration group may be estimated at about 5000 mg/kg bw/day; since the grape extract constituted 40% of the powder, ingestion of the extract was about 2000 mg/kg bw/day and this may be regarded as the NOAEL in this study.

4.2.3. Chronic Oral Toxicity

Ray et al. (2001) fed male B6C3F1 mice weighing 15-20 g (number of mice, age, and mean weight were not reported) Purina Laboratory Rodent Chow containing admixed grape seed extract to provide a daily intake of 0 or 100 mg extract/kg bw/day. The test article was ActiVin® water-ethanol proanthocyanidin extract from grape seeds, shown by HPLC and GC/MS methods to contain 74% oligomeric proanthocyanidins (54% dimers, 13% trimers, and 7% tetramers) as well as "a small amount" of catechin derivatives and other flavonoids. The caging arrangements were not described; the mice had free access to chow and tap water and they were observed 4-6 times per day. Groups (number of mice/group was not reported) of mice were sacrificed every 90 days for 12 months (i.e., at 3, 6, 9, and 12 months), and blood and organ samples (brain, heart, intestine, kidney, liver, lung, and spleen) were collected; tissues were sectioned and examined histopathologically.

The authors reported that no "unusual" deaths were observed (no data on mortality were presented), and no significant differences were seen in body weights, physical appearance, absolute or relative organ weights, or histopathology. The blood measurements showed no significant differences in BUN, ALT, or creatine kinase activity. Hepatic genomic DNA fragmentation was evaluated, with no significant differences between groups. While the authors did not report a NOAEL, the absence of adverse effects of grape seed extract at the only dose tested indicates that the NOAEL was 100 mg/kg bw/day.

In a study conducted in parallel to the one discussed above, Ray et al. (2001) studied the effect of different concentrations of grape seed extract in the diets of female B6C3F1 mice weighing 15-20 g (number, age, and mean weight of the mice were not reported) for 6 months. Caging was not reported; the mice had *ad libitum* access to Herlan Teklad lab chow and tap water. The admixture of grape seed extract in the chow provided doses approximating 0, 100, 250, or 500 mg extract/kg bw/day. Animals were observed "several times" per day. After 180 days, the mice were sacrificed, blood was sampled for biochemical analysis (ALT, BUN, and creatine kinase activity) and samples were taken of brain, lung, liver, spleen, heart, kidneys, duodenum, and pancreas, which were sectioned and examined histopathologically.

The authors reported that there were no significant differences in body weights or physical appearance and no "unusual" deaths. No differences were observed in any of the biochemical measures or in the histopathological assessments of the sampled tissues. A NOAEL was not reported, but since no adverse effects were noted at the highest tested dose, this dose of grape seed extract—500 mg/kg bw/day—may be regarded as the NOAEL.

Based on these chronic studies of oral toxicity, along with the acute oral toxicity study discussed earlier, the authors concluded that "acute and chronic long-term [grape seed extract] exposure to animals does not appear to adversely influence the normal physiology or functioning of any of the vital organs... Thus, the data indicate that [grape seed extract] may serve as a safe antioxidant and health-promoting phytopharmaceutical agent."

4.2.4. Developmental and Reproductive Toxicity

Becci et al. (1983b) studied the effect of grape color extract on reproductive performance through two generations of Sprague-Dawley rats. The test article was Welch's Special Grape Color Powder Type BW-AT derived from fermented grape skin by acidic aqueous extraction; no further information was provided regarding the source, production method, or phenolic content

of the extract. Groups of 25 male and 25 female Sprague-Dawley rats weighing 160-180 g (age and mean weight were not reported) were fed Charles River RMH Formula 3200 with admixture of 0, 7.5, or 15% grape color powder (composed of 40% grape extract and 60% maltodextrin). A fourth group of 15 rats/sex received chow with 9% maltodextrin. Rats were caged individually with chow and water available *ad libitum*.

After 3 weeks, all rats of the F_0 generation were paired; all females not pregnant by day 7 were paired with a different male and, if still not pregnant after another 7 days, with a third male. Females still not pregnant were considered infertile. Pregnant females continued on their assigned diets through mating, gestation, and lactation; they were allowed to deliver the F_1 generation normally. Numbers of pregnant females, pups born alive or dead, and survival were recorded. Each litter was randomly culled to 10 pups (5 of each sex if possible), which were weighed at birth and on days 4 and 21, when they were removed from the dams. Two rats of each sex from each litter with close to mean body weights were selected for a 13-week subchronic feeding study and the remaining offspring were discarded. The F_0 parents were sacrificed and gross necropsies were performed.

The rats selected for the subchronic study remained on the diets assigned to their parents; they were observed daily and feed intake and body weight were recorded weekly. The rats underwent ophthalmic examinations at the beginning and end of the subchronic study and 5 rats/sex/group had blood draws at 6 and 13 weeks for measurement of erythrocyte count, total and differential leukocyte counts, hematocrit, hemoglobin, glucose, BUN, sodium, potassium chloride, total protein, bilirubin, AST, ALT, and ALP. At the end of 13 weeks, male and female rats were paired within groups, remaining on their assigned diets. The F_1-generation dams were allowed to deliver normally; numbers of pregnant females, pups born alive or dead, and survival of the F_2 generation were recorded. Each litter was randomly culled to 10 pups (5 of each sex if possible) on day 4; when the pups were 21 days old they and their parents were euthanized and necropsied. Adrenal glands, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thyroid gland, and uterus of F_1-generation animals were excised and weighed. All animals in the control and high-dose group, and 5 rats/sex/group in the other groups, were histopathologically examined, including the weighed organs and the brain, pituitary gland, spinal cord, eyes, lungs, stomach, pancreas, large and small intestine, urinary bladder, seminal vesicles, lymph nodes, skin, sternum, and grossly abnormal tissues.

Grape color powder fed to pregnant F_0 and F_1-generation rats up to a level of 15% of the diet had no effect on fertility or the number of live-born pups per litter; there were no differences in fertility, gestation, viability, or lactation indices. F_0 and F_1-generation pups receiving grape color powder weighed significantly less than control pups at 21 days after birth; the F_1 animals were significantly lighter at birth, but not at 4 days of age while the pups of the F_0 high-dose group were significantly lighter at 4 days but not at birth. While there were no differences in feed intake, the weight gain of the high-dose F_1-generation female rats in the subchronic study was significantly reduced compared with controls. The authors noted that, "Food conversion data was comparable among groups, thus the decrease in body-weight gain during this phase was most likely the result of the lower calorific value (w/w) of the feed supplemented with the grape colour powder compared with the control feed."

In the subchronic study, no consistent significant effects were observed in hematological or biochemical measures and there were no remarkable ophthalmologic findings. Absolute and

relative liver weights were significantly reduced in male rats receiving diets containing grape color powder, as were absolute adrenal gland weights; reduced absolute adrenal gland weights were also noted in females receiving diets with 15% grape color powder. Male rats receiving grape color powder exhibited significantly reduced relative thyroid gland weights. The only death during the subchronic study was a male rat in the maltodextrin control group. There were no remarkable histopathological findings.

There were no significant differences between groups in feed conversion, and the authors suggested that the decreased body-weight gain was due to the lower caloric value of the feed in diets containing grape color extract. Since the differences in liver, adrenal gland, and thyroid gland weights were unaccompanied by corresponding effects on clinical chemistries or histopathological data, the authors argued that these differences were not indications of toxicity. They concluded that, "No evidence of toxicity or pathological effects in rats fed grape colour powder through two generations was noted." Ingestion of grape color powder at 15% of the diet might be estimated (based on the reported mean bodyweight gains and mean feed conversion) at about 10,000 mg/kg bw/day, and thus the NOAEL for ingestion of grape extract, constituting 40% of the powder, was about 4000 mg/kg bw/day for both reproductive effects and subchronic oral toxicity.

4.2.5. Genotoxicity/Mutagenicity

Brown and Dietrich (1979) performed bacterial reverse mutation assays on more than 70 naturally occurring and synthetic flavonoids using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and TA1538, both with and without S9 activation. While the results were equivocal for quercetin and kaempferol, proanthocyanidin oligomers and monomers exhibited no indications of mutagenicity either with or without S9 activation at the maximum tested concentration of 5000 µg/plate.

Using *S. typhimurium* tester strains TA97, TA98, and TA100, Yu and Swaminathan (1987) assessed potential mutagenicity of a procyanidin polymer, a trimer, and dimers A2, B1, B2, B3, and B4 at concentrations of 500 and 5000 µg/plate in the presence and absence of S9 mix. No evidence of mutagenicity was seen with any tested procyanidin except dimer B4. The cause of the mutagenic effects of B4, however, proved to be due to a contaminant, rutin; when B4 was purified it no longer exhibited any indication of mutagenicity.

Popp and Schimmer (1991) tested induction of sister-chromatid exchanges (SCE), polyploidy, and micronuclei in human lymphocyte cultures obtained from healthy donors by 19 naturally occurring flavonoids: the flavones apigenin, luteolin, luteolin-7-O-glucoside, vitexin, and orientin; the flavonols quercetin, kaempferol, spiracoside, rutin, and hyperoside; the anthocyanidins and flavanols cyanidinchloride, catechin, and epicatechin; and the procyanidin dimers B1, B2, B3, B5, trimer C1, and tetramer D. Compounds were tested at concentrations of 0, 5, 10, 20, 50, 100, 200, and 500 µg/ml, although concentrations higher than 50 µg/ml were not tested if toxicity was observed at that level.

Moderate and statistically significant SCE increases were seen with vitexin and orientin, while weak but still statistically significant increases in SCE were observed at high concentrations of apigenin, luteolin, luteolin-7-O-glucoside, quercetin, spiraeoside, cyanidinchloride, and epicatechin. Significant increases in induction of micronuclei were noted with apigenin, luteolin, luteolin-7-O-glucoside, vitexin, orientin, quercetin, kaempferol,

spiraeoside, and cyanidinchloride. Substantial increases in polyploidy were seen only with procyanidin trimer C1 and tetramer D. No effects on any measure were observed with the flavonol O-glycosides or the monomeric or dimeric flavanols.

In an assessment of the safety of procyanidin dimer B2 as a hair growing agent, Takahashi et al. (1999) evaluated its potential mutagenicity in bacterial reverse mutation, *in vitro* chromosomal aberration, and *in vivo* mammalian micronucleus tests. The Ames assay employed tester strains *S. typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA, with and without S9 metabolic activation, and dimer B2 at concentrations of 156, 313, 625, 1250, 2500, and 5000 µg/plate. Chromosomal aberration was assessed with Chinese hamster liver cells, with and without S9 activation. Micronucleus testing used 8-week-old male Crj:CD-1(ICR) mice that received doses of 500, 1000, or 2000 mg B2/kg bw by subcutaneous injection.

None of the bacterial strains in the reverse mutation test, with or without metabolic activation, showed an increase in revertants, and in the *in vitro* chromosomal aberration test, no structural or chromatid-type aberrations were observed either with or without S9 mixture. However, moderate but statistically significant polyploidy was observed in the activated system only. In the *in vivo* micronucleus test, the frequency of micronucleated polychromatic erythrocytes in mice given procyanidin B2 was not different from the negative control.

Employing a bacterial reverse mutation assay with *S. typhimurium* TA98 and a chromosomal aberration test with V79 Chinese hamster cells, Duarte Silva et al. (2000) evaluated the genotoxicity of catechin and 8 flavonols—galangin, kaempferol, quercetin, myricetin, fisetin, morin, rhamnetin, and rutin. In the absence of S9 mix, only quercetin produced a significant increase in revertant colonies, but morin, galangin, kaempferol, and rhamnetin were also mutagenic with S9 activation. In the chromosomal aberration assay, only kaempferol was genotoxic in the presence of S9 mix but kaempferol, fisetin, quercetin, and myricetin produced significant increased numbers of aberrant cells in the absence of activation. The authors concluded that there is a structure-activity relationship in the genotoxic activity of flavonols, with genotoxicity requiring the presence of a free hydroxyl group at position 5 of the A ring or position 3 of the C ring. They suggested that the observed genotoxic effects are due to auto-oxidation rather than direct genotoxicity of the flavonols.

Yamakoshi et al. (2002) tested the genetic toxicity of grape seed extract in 3 tests, a bacterial reverse mutation assay, chromosome aberration test with Chinese hamster lung cells, and mouse micronucleus test. The extract, prepared in the laboratory, was derived from grape seeds by water-ethanol extraction, condensed, filtered, and spray-dried. Based on HPLC, the content of the extract was estimated as 89.3% proanthocyanidins (6.6% dimers, 5.0% trimers, 2.9% tetramers, and 74.8% oligomers and polymers), 6.6% monomeric flavanols [2.5% (+)-catechin, 2.2% (-)-epicatechin 1.4% (-)-epigallocatechin, and 0.5% (-)-epigallocatechin gallate], 2.24% moisture, 1.06% protein, and 0.8% ash.

The Ames assay was conducted with *S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537, both with and without S9 activation, at concentrations of 0, 19, 39, 78, 156, 313, 625, and 1250 µg/plate in the first two strains and 0, 156, 313, 625, 1250, 2500, and 5000 µg/plate in the last two. (The reduced concentrations were used with TA98 and TA100 because the grape seed extract inhibited the growth of these strains.) No increase in the number

of revertant colonies occurred in any of the 4 test strains at any concentrations of grape seed extract, either in the presence or absence of S9 mix.

Grape seed extract was examined for its potential to induce structural chromosome aberrations and aneuploidy or polyploidy in cultured Chinese hamster lung cells with and without S9 mixture. Cells were exposed for 6 hours at doses of 0, 18.8, 37.5, and 75.0 µg/ml and for 24 and 48 hours at doses of 0, 9.4, 18.8, and 37.5 µg/ml in the absence of S9, and for 6 hours at doses of 0, 18.8, 37.5, 75.0, 150.0, and 300.0 µg/ml in the presence of S9. Procyanidin dimers, trimers, and tetramers were separated from grape seed extract and were also evaluated in the Chinese hamster chromosome aberration test. No statistically significant increases in the frequency of metaphases with aberrant chromosomes were seen at either sampling time, either in the presence or absence of S9. Aneuploidy or polyploidy was also not observed in the test. Procyanidin dimers, trimers and tetramers did not cause aneuploidy or polyploidy in either the activated or non-activated system.

For the mammalian micronucleus test, 8-week-old male ddY mice received grape seed extract in 2 doses of 0, 500, 1000, or 2000 mg/kg bw separated by 24 hours, with 5-6 mice/dose. The frequencies of micronucleated peripheral reticulocytes were not significantly different between the grape skin extract groups and the control group.

The results of all three tests demonstrated that, under the conditions tested, neither grape seed extract nor procyanidin dimers, trimers, and tetramers were mutagenic.

Erexson (2003) assessed the potential for clastogenic activity of two grape extracts in mouse micronucleus assays conducted in compliance with OECD Guideline 474. The test articles were MegaNatural™ grape seed extract (containing 90.5% [w/w] total phenols, 4.8% catechin, 4.4% epicatechin, and 1% gallic acid, with no detectable anthocyanins; the extract included 10.4% monomers, 74.9% oligomers, and 14.7% polymers) and Meganatural™ grape skin extract (containing 87.3% [w/w] total phenols, 3.4% catechin, 4.6% epicatechin, 2.4% gallic acid, and 2.6% total anthocyanins; including 16.7% monomers, 67.4% oligomers, and 15.9% polymers). Young adult male Crl:CD-1® (ICR) BR mice were maintained in polycarbonate cages with 6 mice/cage. Test groups for each condition (test article, vehicle control, and positive control at each bone-marrow-harvest time point) included 5 mice, with 1 reserve mouse for any mice lost during treatment. The test vehicle was 0.5% aqueous carboxymethyl cellulose administered in a gavage volume of 20 ml/kg bw. Doses were 0, 500, 1000, and 2000 mg/kg bw to mice scheduled for the 24-hour harvest and 0 and 2000 mg/kg bw for the 48-hour time point. At the scheduled time point, the animals were euthanized and marrow was extracted from tibias; marrow slides were scored for micronuclei and the PCE:NCE cell ratio.

One grape seed extract high-dose animal died from gavage error, but there was no other mortality and no signs of clinical toxicity. Grape seed extract was cytotoxic to the bone marrow at the 2000 mg/kg bw high-dose level as shown by a significant decrease in the PCE:NCE ratio at the 48-hour harvest time point (0.44 as compared to 0.61, 0.76, and 0.55 for doses of 500, 1000, and 2000 mg/kg bw, respectively, at 24 hours), but no statistically significant increase in the numbers of micronucleated cells was observed at any dose or harvest time point. The grape skin extract induced no signs of clinical toxicity, was not cytotoxic, and did not increase the numbers of micronucleated cells at any dose or time point. The authors concluded that, "The results of this assay demonstrate that Meganatural™ [grape seed extract] and [grape skin extract]

are devoid of clastogenic activity when administered orally to mice at doses as high as 2000 mg/kg."

The National Toxicology Program (NTP) performed an Ames assay on a grape seed extract sold in the U.S. under the name Masquelier's™ Original OPCs (Oligomeric Proantho-Cyanidins), although the NTP noted that, "Based on the structures of identified active ingredients, the phenolic compounds extracted from grape seeds ... would not be expected to be genotoxic" (NTP 2000). The NTP also stated that the tested product is "representative of dietary supplements with activity based on OPCs." Following its standard protocol for Ames tests, the NTP tested the extract using tester *S. typhimurium* strains TA97, TA98, TA100, and TA1535 both without metabolic activation and with S9 derived from both rat and hamster livers at both 10% and 30% concentration. The extract was tested at concentrations of 0, 100, 333, 1000, 3333, and 10000 µg/plate and revertant colonies were counted after 2 days. No significant increase was seen relative to the negative control (water), and the NTP concluded that grape seed extract is not mutagenic under the conditions of this test system (NTP 2003).

Aiub et al (2004) studied genotoxic effects of a grape skin extract prepared in the laboratory. Isabel varietal grapes (*V. labrusca*) were washed and the skins separated and boiled in distilled water. Ethanol was added to the decoction and the mixture was stored for 20 days, filtered, subjected to vacuum evaporation, and lyophilized. Each 100 g wet grape skin yielded about 8.9 g extract; HPLC analysis indicated a total phenolic content of 103 mg/g (10.3%) with proanthocyanidin content of 55 mg/g and detected levels of gallic acid, catechin, epicatechin gallate, resveratrol, rutin, and other unidentified flavanoids.

The extract was tested in an Ames assay with *S. typhimurium* strains TA97, TA98, TA100, and TA102 at concentrations of 0, 0.1, 1, 10, and 100 µg/ml, with and without S9 metabolic activation prepared from the liver of Sprague-Dawley rats. The same concentrations of extract, with and without S9, were used for an SOS chromotest using *Escherichia coli* strains PQ65, PQ66, OG40, and OG100 (Quillardet et al. 1982). Finally, a comet assay was performed using 3T3 fibroblasts harvested from Balb/c mice to evaluate DNA breakage.

Although cytotoxicity was observed in tester strains TA97 and TA102 at concentrations ≥0.1 µg/ml, no mutagenicity was observed in any strain at any tested concentration with or without S9 activation. No significant increase in the induction of the SOS function *sfiA* was seen, indicating an absence of DNA damage in the exposed *E. coli* cells. DNA breakage in the comet test was not significantly increased by exposure to grape skin extract. The authors concluded that their data suggest that grape skin extract is not mutagenic.

4.2.6. Conclusions Regarding Toxicity

A variety of grape seed extracts, grape skin extracts, and individual phenolic compounds derived from grape extract has been subjected to extensive toxicological evaluation. Published toxicity research include 4 studies of acute toxicity (3 oral, 1 subcutaneous), 5 subchronic feeding studies in rats and dogs, 2 chronic feeding studies in mice, a 2-generation developmental/ reproductive toxicity study in rats, and a large battery of studies of genetic toxicity: 7 bacterial reverse mutation assays, a study of sister-chromatid exchange, 1 *in vitro* and 4 *in vivo* studies of micronucleus induction, 2 polyploidy assays, and 3 *in vitro* tests of chromosomal aberration. All of the tested extracts are generally similar with regard to phenolic composition, although they may differ in the relative abundance of individual polyphenols, and so it is appropriate to use

these data to assess the safety of the intended use of exGrape® Total. The strength of this evidence is enhanced by the variety of test articles employed with similar findings.

While some of the genotoxicity studies reported isolated instances of equivocal or positive findings, most studies were negative and it was suggested that the few positive results may be due to auto-oxidation rather than direct genotoxicity (Duarte Silva et al. 2000). In at least one case, an apparent positive finding of mutagenesis (of dimer B4) proved to be caused by a rutin contaminant, and no evidence of mutagenicity was seen once the B4 was purified.

Acute oral administration of grape seed extract to albino rats at up to 5000 mg/kg bw or to Fischer 344/DuCrj rats at up to 4000 mg/kg bw produced no toxicity (Ray et al. 2001; Yamakoshi et al. 2002). At an acute oral dose of 12,650 mg grape seed extract/kg bw, reduced feed intake and increased mortality was seen (El-Adawi et al. 2006), but no adverse effects were apparent at doses of 6300 mg/kg bw or less.

In all 5 subchronic feeding studies, each using a different extract, all substantially similar to exGrape® Total, the NOAEL was the highest dietary concentration tested:

- MegaNatural™ grape seed extract at 2.5% in the diets of male and female Sprague-Dawley rats (20 rats/sex/dose), equivalent to 2150 and 1780 mg/kg bw/day for females and males, respectively (Bentivegna and Whitney (2002)
- MegaNatural™ grape skin extract at 2.5% in the diets of male and female Sprague-Dawley rats (20 rats/sex/dose), equivalent to 2150 and 1780 mg/kg bw/day for females and males, respectively (Bentivegna and Whitney (2002)
- IH636 grape seed extract at 2.0% in the diets of male and female Sprague-Dawley rats (20 rats/sex/level), equivalent to 1928 and 1586 mg/kg bw/day for females and males, respectively (Wren et al. 2002)
- Laboratory-prepared grape seed extract at 2.0% in the diets of male and female Fischer 344/DuCrj rats (10 rats/sex/level), equivalent to 1501 and 1410 mg/kg bw/day for females and males, respectively (Yamakoshi et al. 2002)
- Welch's Special Grape Color Powder Type BW-AT (40% grape skin extract) at 15.0% in the diets of male and female beagle dogs (4 dogs/sex/level), equivalent to approximately 5000 mg powder (2000 mg grape skin extract)/kg bw/day (Becci et al. 1983a)

The two published chronic feeding studies, conducted by Ray et al. (2001), tested yet another grape seed extract similar to exGrape® Total, ActiVin®, which was given to male B6C3F1 mice at a dose of 100 mg/kg bw/day for 12 months and to female B6C3F1 mice at doses up to 500 mg/kg bw/day for 6 months with no indications of toxicity.

The single published study of reproductive toxicity (Becci et al. 1983b) involved the dietary admixture of Welch's Special Grape Color Powder Type BW-AT (40% grape skin extract) at up to 15% dietary concentration in the diets of F_0-generation Sprague-Dawley rats (25 rats/sex/dose) for 3 weeks before mating and through lactation, to the F_1 pups for 13 weeks before mating and through lactation, and to the F_2 generation pups for 21 days. No adverse effects were seen on any endpoint, and the NOAEL for both reproductive effects and subchronic oral toxicity was the highest level tested, equivalent to about 4000 mg grape seed extract/kg bw/day.

In vitro studies of the genotoxicity of individual components of grape seed extract have been inconsistent, often finding no evidence of mutagenicity or clastogenicity but occasionally producing equivocal or positive results. In one such study, the authors suggested that the observed genotoxic effects were due to auto-oxidation rather than direct genotoxicity. No *in vitro* tests of grape seed extract itself, such as the NTP studies, have found any indication of mutagenicity, and neither grape seed extract nor any of its components has produced positive findings in *in vivo* tests of genotoxicity.

In summary, these studies show that the extracts of grape seed or grape skin tested are not toxic to experimental animals at levels as high as 6% of the diet for extended periods of time, nor did they cause adverse reproductive or developmental effects. This evidence is particularly strong because a wide variety of products has been tested, differing not only in total phenolic content but also in the distribution of chain lengths of polymers and oligomers as well as the prevalence of tetramers, trimers, dimers, and monomers as well as in the proportions of proanthocyanins and specific polyphenolic compounds.

Table 14. Toxicity Studies.

Reference	Test Article	Animal Model	Dose and Administration	Duration	Result
Acute Oral Toxicity					
El-Adawi et al. (2006)	Lab-prepared grape seed extract	M Wistar rats	0, 3000, 4630, 6380, 9000, or 12650 mg/kg bw by gavage	single dose	Reduced feed intake & increased mortality at highest doses; LD_{50} (probit analysis) = 6300 mg/kg bw
Ray et al. (2001)	ActiVin® grape seed extract	M & F albino rats (strain not reported)	0 or 5000 mg/kg bw by gavage	single dose	No toxicity seen; LD_{50} > 5000 mg/kg bw
Yamakoshi et al. (2002)	Lab-prepared grape seed extract	M & F Fischer 344 rats	0, 2000, or 4000 mg/kg bw by gavage	single dose	No toxicity seen; LD_{50} > 4000 mg/kg bw
Repeated-Dose Oral Toxicity					
Becci et al. (1983a)	Welch's Special Grape Color Powder Type BW-AT (40% grape extract in maltodextrin)	M & F beagle dogs	0, 3, or 6% grape extract feed admixture	90 days	No toxicity seen; NOAEL = 6% dietary concentration ≈ 2000 mg grape extract/kg bw/day
Becci et al. (1983b)	Welch's Special Grape Color Powder Type BW-AT (40% grape extract in maltodextrin)	M & F Sprague-Dawley rats from the F_1 generation of a study of reproductive performance	0, 3, or 6% grape extract feed admixture	90 days	No toxicity seen; NOAEL = 6% dietary concentration ≈ 4000 mg grape extract/kg bw/day
Bentivega and Whitney (2002)	MegaNatural™ grape seed extract	M & F Sprague-Dawley rats	0, 0.625, 1.25, or 2.5% feed admixture	90 days	No toxicity seen; NOAEL = 2.5% dietary concentration = 2150 and 1780 mg/kg bw/day in F & M rats
Bentivega and Whitney (2002)	MegaNatural™ grape skin extract	M & F Sprague-Dawley rats	0 or 2.5% feed admixture	90 days	No toxicity seen; NOAEL = 2.5% dietary concentration = 2150 and 1780 mg/kg bw/day in F & M rats
Ray et al. (2001)	ActiVin® grape seed extract	M B6C3F1 mice	0 or 100 mg/kg bw/day as feed admixture	12 months	No toxicity seen; NOAEL = 100 mg/kg bw/day in M rats
Ray et al. (2001)	ActiVin® grape seed extract	F B6C3F1 mice	0, 100, 250, or 500 mg/kg bw/day as feed admixture	6 months	No toxicity seen; NOAEL = 500 mg/kg bw/day in F rats
Wren et al. (2002)	IH636 grape seed water extract	M & F Sprague-Dawley rats	0, 0.5, 1.0, or 2.0% feed admixture	90 days	No toxicity seen; NOAEL = 2.0% dietary concentration = 1928 and 1586 mg/kg bw/day in F & M rats
Yamakoshi et al. (2002)	Lab-prepared grape seed extract	M & F Fischer 344 rats	0, 0.02, 0.2, or 2.0% feed admixture	90 days	No toxicity seen; NOAEL = 2.0% dietary concentration = 1928 and 1586 mg/kg bw/day in F & M rats
Reproductive Toxicity					
Becci et al. (1983b)	Welch's Special Grape Color Powder Type BW-AT (40% grape extract in maltodextrin)	M & F Sprague-Dawley rats in F_0 and F_1 generations	0, 3, or 6% grape extract feed admixture	F_0: 6-8 weeks to parturition F_1: ~16 weeks to parturition	No adverse effects seen on reproductive performance; NOAEL = 6% dietary concentration ≈ 4000 mg grape extract/kg bw/day

4.3. Other Animal Studies

The studies discussed below are summarized in Table 15 at the end of the section.

4.3.1. Mice

Bagchi et al. (2001) studied the ability of grape seed extract to protect against drug- and chemical-induced multiorgan toxicity in mice. The test article was ActiVin® water-ethanol proanthocyanidin extract from grape seeds, shown by HPLC and GC/MS methods to contain 74% oligomeric proanthocyanidins (54% dimers, 13% trimers, and 7% tetramers) as well as "a small amount" of catechin derivatives and other flavonoids. The animals used were 3-month-old male ICR (CD-1) mice weighing 30-40 g (the mean weight was not reported). Mice had free access to Purina Laboratory Rodent Chow and tap water; caging arrangements were not reported. Toxicities of 6 agents were investigated in 6 parallel experiments. Each experiment was a 2x2 factorial design with groups that received saline by gavage (control), 100 mg grape seed extract/kg bw/day by gavage and no toxic agent, toxic agent alone (intraperitoneally), or grape seed extract followed by toxic agent. Numbers of mice assigned to each group were not reported, but (based on table footnotes) appears to have been 5-9. The mice received grape seed extract for a number of days prior to toxic-agent exposure as follows:

- 7 days prior to 500 mg/kg bw dose of acetaminophen (inducing hepatotoxicity)
- 10 days prior to 200 mg/kg bw dose of amiodarone (inducing pulmonary toxicity)
- 9 days prior to 20 mg/kg bw dose of doxorubicin (inducing cardiotoxicity)
- 7 days prior to 12.5 mg/kg bw dose of cadmium chloride (inducing nephrotoxicity)
- 8 days prior to 10 mg/kg bw dose of dimethylnitrosamine (inducing splenotoxicity)
- 8 days prior to 200 µl/kg bw dose of *O*-ethyl-S,S-dipropyl phosphorodithioate (MOCAP; inducing neurotoxicity)

Measured endpoints were serum ALT, BUN, and creatine kinase activity; counts of normal, apoptotic, and necrotic cells in target tissues; and DNA fragmentation in target tissues.

No effects of ingestion of grape seed extract alone were observed in any endpoint in any of the experiments. The 6 agents inoculated alone consistently produced the expected toxic effects, which were significantly reduced or abolished by pretreatment with grape seed extract.

Using a mouse model of Alzheimer's disease, Wang et al. (2008) studied the effect of MegaNatural™ grape seed polyphenolic extract (which contains about 8% monomers, 75% oligomers, and 17% polymers, with epicatechin the most abundant oligomer) on aggregation of amyloid β-protein into high-molecular-weight Aβ oligomers. Adult female Tg2576 AD transgenic mice were treated for 5 months, beginning at 6 months for behavior testing and at 10 months for neuropathology and mechanistic study, during which they received *ad libitum* either pure drinking water or water supplemented with 200 mg grape seed extract/kg bw/day (approximately equivalent to human exposure of 1 g/day). Spatial memory was assessed by a water-maze test; after sacrifice, brains were harvested for measurement of total Aβ, soluble Aβ oligomers, amyloid precursor protein, and α-, β-, and γ-secretase activity.

Mice receiving grape seed extract performed significantly better than controls in the water-maze test and showed significantly less oligomerization of Aβ peptides into high-

molecular-weight species and significant reduction of total and soluble Aβ levels, but no change in amyloid precursor protein or in α-, β-, or γ-secretase activity. The authors suggested that the grape seed extract "might exert its beneficial effect *in vivo* primarily through the prevention of Aβ oligomerization into soluble high-molecular-weight species." With regard to safety, the authors reported that:

> "Consistent with previous clinical and experimental evidence..., [grape seed extract] treatment delivered in the drinking water for 5 months did not result in detectable adverse effects, including changes in body weight or water consumption. Normal liver functions were observed in both [grape seed extract]-treated and water-treated control groups, as reflected by normal serum levels of aspartate aminotransferase and alanine aminotransferase."

Wen et al. (2008) studied the ability of a grape seed extract containing ≥85% procyanidins to inhibit tumor formation in 6-8-week-old SCID mice. The mice were gavaged with 100 µL water containing either 0 (n = 5) or 50 (n = 6) mg/kg bw grape seed extract, followed by an injection of breast-cancer cells; the mice continued to be gavaged daily with water or grape seed extract while tumor volume was assessed twice a week and body weight was measured weekly for 11 weeks. Treatment with grape seed extract significantly inhibited tumor growth and the "treatment seemed to have no obvious toxicity and showed no detectable effect on body weight and behavior of mice."

Using a commercially produced extract of red grape pomace referred to as oenocyanin E163, Cai et al. (2010) tested the effect of an anthocyanin-rich extract on adenoma development in the Apc^{Min} mouse, a model for human familial adenomatous polyposis coli. The test article had a anthocyanin concentration of 22% (w/w), including malvidin-3-glucoside (40%), peonidin-3-glucoside (20%), cyanidin-3-glucoside (16%), petunidin-3 glucoside (12%), and delphinidin-3-glucoside (7%); the extract also contained about 12% flavan-3-ols. Apc^{Min} mice were assigned to receive AIN-93G chow from week 4 to week 16 either with or without 0.3% (w/w/) extract supplementation (n = 13-14 mice/group). After sacrifice, the intestinal tracts of the mice were removed and examined for adenomas.

Supplementation with grape pomace extract significantly reduced adenoma development by about 50% with no reported adverse effects. In a pharmacokinetic side experiment, plasma, urine, and intestinal mucosa of mice which had received the extract were analyzed for anthocyanins, which were identified in urine and mucosa but not plasma. The authors regarded their findings as supportive of a role for anthocyanin-rich grape pomace extract in chemopreventive intervention.

Wang et al. (2010) employed a rat model to study the ability of grape seed extract to modulate the onset and progression of Huntington's disease. The test article was MegaNatural™ grape seed polyphenolic extract, which contains about 8% monomers, 75% oligomers, and 17% polymers, with epicatechin the most abundant oligomer. R6/2 ovarian-transplant female mice (number, age, and weight were not reported) were randomly assigned to receive 100 mg/kg bw/day of pure water or grape seed extract and were then mated with wild-type male mice; pups received the same treatment as their parents. Motor performance in the pups was assessed by their ability to balance on an accelerating rotating rod at 6, 9, and 11 weeks of age.

No difference in performance was seen at 6 weeks, but at 9 and 11 weeks the mice receiving grape seed extract had significantly better motor coordination than the controls. Grape

seed extract also significantly extended the median lifespan of the rats from 90.5 days to 98 days. The authors noted at the treatment with grape seed extract was "well tolerated," with no adverse effects apparent.

Using the same MegaNatural™ grape seed polyphenolic extract as in the previous study (Wang et al. 2010), Santa-Maria et al. (2011) assessed the effect of such extracts on the intracellular accumulation of hyperphosphorylated tau protein in the spinal cords of 9-month-old male JNPL3 transgenic mice expressing a human tau protein containing the P301L mutation. Seven mice received 150 mg grape seed extract/kg bw/day in their drinking water for 6 months while 9 matched mice received pure water. Motor function was assessed through a wire-hang test; after sacrifice brains and spinal cords were removed for histochemical examination.

The authors stated that "Long-time [grape seed extract] treatment for 6 months was well tolerated as reflected by normal grooming behavior and steady body weight values." The treatment significantly reduced levels of hyperphosphorylated and conformationally modified tau in spinal-cord tissue and significantly improved neuromuscular function, and the authors concluded that the results "suggest that [grape seed extract] can interfere with tau-mediated neurodegenerative mechanisms and ameliorate neurodegenerative phenotype in an animal model of tauopathy ... [and] support further evaluation of [grape seed extract] for preventing and/or treating of tauopathies in humans."

4.3.2. Rats

Tebib et al. (1994) studied the effect of consumption of grape seed extract on lipids and lipases in male Sprague-Dawley rats fed hypercholesterolemic diets. The extract was purified to contain ~100% procyanidins, including 37% tetra- to octameric, 21% trimeric, 31% dimeric, and 11% monomeric; monomers and polymers were then separated. Forty-eight rats weighing about 190 g were individually housed in metabolic cages and divided into 4 groups with n = 12 rats/group. One group, the control, received a diet of standard rat chow while a second group received chow with 38% saturated fatty acids and 1% cholesterol. The remaining groups were fed the hypercholesterolemic diet supplemented by either 2% procyanidin monomers or polymers. Half of the animals from each group were examined at 3 and 9 weeks. Blood was taken from the heart, liver and epididymal fat pads were collected, the aorta was excised, and feces from the last 5 days were analyzed.

At both 3 and 9 weeks, the rats receiving procyanidin polymers had the lowest levels of TC, LDL- and VLDL-cholesterol, and the highest levels of HDL-cholesterol, all significantly different from the rats receiving the hypercholesterolemic diet without grape seed extract; the rats with monomer supplementation were between the other groups. There was no difference between groups in body protein, but body fat was significantly lower in the polymer-supplemented group, as was lipoprotein lipase activity. The authors concluded that "dietary polymeric grape seed tannins reduce plasma total cholesterol, triacylglycerol, and LDL cholesterol concentrations in high cholesterol fed rats," with no adverse effects reported from consuming monomers or polymers at 2% dietary concentration for 9 weeks.

In a study with male Sprague-Dawley Rats weighing about 145 g, Tebib et al. (1996) studied the effect of grape seed extract on colonic bacterial enzymes and cecal fermentation. The extract was purified to contain ~100% procyanidins, including 37% tetra- to octameric, 21% trimeric, 31% dimeric, and 11% monomeric; monomers and polymers were then separated. The

rats were individually housed in metabolic cages and divided into 3 groups of n = 6 rats/group, which received standard rat chow or rat chow supplemented with 0.71% procyanidin monomers or polymers. This addition level was chosen to approximate the procyanidin load of about 250 mg that a human adult would achieve from consumption of 500 ml red wine. The animals remained on their assigned diets for 12 weeks, then sacrificed and the cecum and cecal contents were examined. The colon was excised and assayed for bacterial enzyme activity (β-glucosidase, β-glucuronidase, mucinase, and nitroreductase) and the fecal colonic content tested for nitrogen.

There were no differences in feed intake, but the rats fed chow supplemented with procyanidin polymers gained significantly less weight than rats in the other 2 groups. The rats receiving polymers also had significantly thinner cecal walls, higher concentrations of volatile fatty acids (levels of acetic, propionic, and butyric acids were all significantly higher), and consequently lower pH cecal contents than the other groups. The polymer group had higher nitrogen content of colonic fecal matter. There was no difference between groups in overall level of colonic bacterial enzymes, but if enzyme activity was expressed in proportion to nitrogen (i.e., protein) content, activity of β-glucosidase and mucinase was significantly lower in rats that had ingested procyanidin polymers. The authors concluded that "polymeric grape seed tannins intake lead to a stimulation of fermentative activities [reflected in the increased concentrations of volatile fatty acids in the cecum] without an increase of deleterious enzymic activities."

In a study of the effects of red grape seed proanthocyanidins on the recovery of postischemic function in isolated rat hearts, Pataki et al. (2002) employed the commercial product ActiVin, which contains 54% dimeric proanthocyanidins, 13% trimers, 7% tetramers, and <5% each of monomers and high-molecular-weight oligomers and polymers. Male and female 17-19-week-old Sprague-Dawley rats weighing 320-340 g were housed 2 rats/cage and given free access to chow and water. After 3 days of acclimatization, the rats were divided into 3 groups (n = 12 rats/group) and given daily gavage doses of 0, 50, or 100 mg extract/kg bw in 10 ml water/kg bw for 3 weeks. After sacrifice the rats' hearts were excised, subjected to 30 minutes of ischemia, and given 2 hours of reperfusion. Coronary effluents were collected and the concentration of oxygen free radicals was measured.

Ingestion of grape seed extract resulted in a significant dose-dependent reduction in the production of oxygen free radicals. The treatment had no effect on feed intake or body weight, and no adverse effects were reported.

Al-Awwadi et al. (2005) studied the capacity of 3 different grape seed extracts to prevent hypertension, cardiac hypertrophy, increased production of reactive oxygen species, and increased expression of cardiac NADPH oxidase in a rat model of insulin resistance. Sprague-Dawley rats weighing 185-220 g were divided into 5 groups (n = 9 rats/group) and housed 3 rats/cage. One group received standard chow as a control, while the other 4 groups were given feed enriched with 60% fructose to induce insulin resistance. Three of the high-fructose groups received a daily gavage of 10 ml grape seed extract/kg bw, one extract enriched in anthocyanins, one enriched in procyanidins and galloylated procyanidins, and one rich in catechin oligomers. All treatments provided 2421 mg polyphenols/kg bw/day, calculated to be the daily equivalent of a 70-kg human consuming 500 ml red wine containing 1500 mg total polyphenols . Treatment continued for 6 weeks, during which the rats had free access to feed and drinking water. Intake of feed and water were recorded daily; twice a week the animals were weighed and their blood pressure was assessed. At the end of the in-life study, blood was collected and analyzed for

glucose, insulin, AST, ALT, BUN, TC, HDL, TG, and phospholipids, and the thoracic aorta and heart were removed for weighing and tissue analysis of superoxide anion production and immunoreactivity.

There was no mortality and no significant differences in body weight, AST, ALT, BUN, blood glucose, and insulin. The rats receiving the high-fructose diet had significantly elevated TG and insulin resistance compared to controls, which were fully reversed by the grape seed extract high in procyanidins and partially reversed by the other extracts. None of the extracts reduced the elevations in phospholipids or LDL induced by the high-fructose diet, but all of them significantly lowered the induced hypertension, cardiac hypertrophy, and increase superoxide-anion production. The authors concluded that "polyphenolic extracts enriched in different types of polyphenols possess differential effects on insulin resistance, hypertension, cardiac hypertrophy, and hyperlipidemia." All of the effects of the 3 grape seed extracts were regarded as beneficial, with no reported adverse effects.

Using a rat model, El-Adawi et al. (2006) investigated the ability of grape seed extract to prevent the development of hypercholesterolemia or to reduce already-developed hypercholesterolemia. The test article was prepared by extracting dried ground grape seeds with aqueous ethanol for 120 minutes at 70°C, resulting in an extract containing 598.8 mg polyphenols/g. Forty-eight male Wistar rats aged 35 days and weighing 100-120 g were assigned to 3 groups: Group 1 (n = 24) was fed a high-cholesterol diet (not further described), Group 2 (n = 12) received the high-cholesterol diet supplemented with 0.3% grape seed extract, and Group 3 (n = 12) received normal rat chow. Lipid profiles in Group 1 were monitored until the development of hypercholesterolemia, at which time the group was divided into 2 groups of n = 12 which received rat chow enriched with either 0 or 0.3% grape seed extract.

All of the rats in Group 1 developed hypercholesterolemia within 8 weeks, with total cholesterol concentrations more than double those of the control group and LDL-cholesterol levels more than 5 times those of controls. Supplementation with 0.3% grape seed extract did not fully prevent the development of hypercholesterolemia, but total and LDL-cholesterol were significantly lower than in the unsupplemented rats, by 32% and 40%, respectively. Grape seed extract also significantly reduced both total and LDL-cholesterol in the Group-1 rats that had already developed the disease, lowering them by 42% and 56%, respectively. No data regarding feed intake were reported, and consequently the ingested dose of grape seed extract cannot be determined. The authors did not report any adverse effects on rats consuming 0.3% grape seed extract for 8 weeks or longer.

Cetin et al. (2008) studied the protective effect of grape seed extract on radiation-induced oxidative stress in the liver. The tested grape seed extract was prepared in the laboratory by ether and ethanol extraction from dried seeds derived from ripened Okuzgozu grapes; its total phenolic content (gallic acid equivalents) was about 57%. Forty-eight male Wistar rats (age not reported) weighing 240-320 g were divided into 4 groups (n = 12 rats/group) and given free access to feed and water; housing was not described. Two groups received a daily gavage providing 100 mg grape seed extract/kg bw for 11 days while rats in the other 2 groups received only water. On day 7, one of the groups of rats receiving each treatment was exposed to 8 Gy whole-body irradiation. At the end of day 11 the rats were sacrificed, blood was collected, and livers were excised and homogenized and assayed for malondialdehyde, superoxide dismutase, catalase, and protein.

Analysis of blood showed that among the rats that did not receive radiation, there was no difference between those ingesting grape seed extract and the controls in white and red blood cell counts, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet count, or lymphocyte count. Radiation significantly depressed white and red blood cell counts, hematocrit, and lymphocyte count and increased the mean cell hemoglobin and mean cell hemoglobin concentration, but the treatment and control groups receiving radiation did not differ from each other. Similarly, although radiation significantly increased malondialdehyde and decreased superoxide dismutase and catalase, and ingestion of grape seed extract provided a significant protective effect, there was no difference between the test and control groups not receiving radiation. No adverse effects were reported due to ingestion of 100 mg grape seed extract/kg bw/day.

The effect of red grape skin extract on rat serum antioxidant capacity was evaluated by Lionetto et al. (2011) in 2 experiments with male Wistar rats weighing 200-250 g. The tested extract was a non-commercial anthocyanin preparation containing 50% malvidin 3-*O*-glucoside, 10% petunidin 3-*O*-glucoside, 8% delphinidin 3-*O*-glucoside, and 5% cyanidin 3-*O*-rutinoside; reportedly, 1 g grape skin yields 160 mg extract. In the first experiment with 16 rats divided into 2 groups of 8, the animals received oral gavage providing 0 or 0.6 mg grape skin extract/kg bw/day for 10 days. In the second experiment, 32 rats were assigned to 4 groups (n = 8 rats/ group) in a 2x2 factorial design: 2 groups received 0.6 mg grape skin extract/kg bw/day for 10 days while the other 2 groups received only pure water, and one group from each dosing regimen was exposed on day 8 to a subcutaneous injection of carbon tetrachloride to produce oxidative stress. All animals from both experiments were sacrificed and blood was taken for analysis of antioxidant capacity.

The authors estimated that the tested dose of 0.6 mg extract/kg bw/day equates to about 25% of an average daily intake of anthocyanins by humans. In the first experiment, the daily gavage with grape skin extract produced a statistically significant 50% increase in antioxidant capacity. The second experiment showed that ingestion of the grape skin extract was capable of completely offsetting the prooxidant effect of carbon tetrachloride administration. No adverse effects were reported. Additionally, in an *in vitro* study with excised segments of rat jejunal mucosa, Lionetto et al. (2011) showed that direct contact of the grape skin extract with the luminal surface of the mucosa for 3 hours resulted in no effect on its permeability or integrity. The authors concluded that "anthocyanin extract from red grape skin enhances the total antioxidant capacity of the serum in either normal physiological condition or during oxidative stress induction."

Dal-Ros et al. (2012) gave 51-week-old male Wistar rats drinking water containing 100 mg red wine polyphenols/kg bw/day (n = 5), water with 3% ethanol (n = 6), or water with 100 mg apocynin/kg bw/day (n = 4) for 4 weeks. The phenolic extract was derived from red wine and dried in the laboratory and was analyzed as containing 47.1% total phenolic compounds expressed as gallic acid. After sacrifice the mesenteric artery rings were excised for measurement of tension and cryosectioned for analysis of endothelial NO synthase, 3-nitrotyrosine, arginase, AT2 receptor, NADPH oxidase, and reactive oxygen species and the results were compared with those from untreated 12-week-old rats that served as a reference group.

Significant improvement was seen in age-related endothelial dysfunction of the mesenteric artery and oxidative stress in rats receiving red wine polyphenols or apocynin. The

authors concluded that "intake of red wine polyphenols, besides retarding the development of an age-related endothelial dysfunction, also improves an established aging-related endothelial dysfunction in the middle-aged rat. The curative effect seems to involve mainly the normalization of vascular oxidative stress due, at least in part, to the reduction of the overexpression of NADPH oxidase and the angiotensin system." No adverse effects were reported from the treatment.

4.3.3. Hamsters

Auger et al. (2002) prepared an extract from red wine and measured its phenolic content by HPLC. The overall phenol content was 471 mg/g, including 8.6 mg catechin/g; 8.7 mg epicatechin/g; 6.9 mg, 8.0 mg, 20.7 mg, and 0.7 mg/g of dimers B1-B4, respectively; 12.4 mg anthocyanins/g; and 20.0 mg phenolic acids/g. Thirty-two male golden Syrian hamsters weighing 60-80 g (age was not reported) were divided into 4 groups of 8, caged by group, and given free access to feed and water. The chow was supplemented with lard and depleted of vitamins C and E and selenium to produce an atherogenic diet. The animals consumed this diet for 8 weeks while receiving via gavage 30.4 mg phenolic extract/kg bw/day along with 7.14 mL/ kg bw/day of either pure water (Group 1) or water with 2.6 mol ethanol/L (Group 2), while Groups 3 and 4 received only pure water or water with ethanol with no phenolic extract.

The hamsters were weighed and food disappearance was measured daily. After 8 weeks the animals were fasted for 18 hours, anesthetized, and subjected to a blood draw. After sacrifice, livers were excised for determination of Se-dependent glutathione peroxidase activity and aortas were excised and dissected for examination of epithelial tissue. Blood was analyzed for TC, HDL, VLDL and LDL, TG, apolipoprotein A-1 and B, and plasma total antioxidant activity (TAC).

Feed consumption differed little among the groups (although it was statistically significantly higher by Group-1 hamsters than the other groups) and there were no significant differences in final body weights. Hamsters that received phenolic extract had significantly reduced TC, heightened TAC, and reduced aortic fatty streaks, but TG, glutathione peroxidase activity and Apo-A1 did not differ among groups. Phenolic extract had no effect on HDL, but it was reduced by ethanol. No adverse effects associated with administration of phenolic extract were noted. The authors concluded that phenolic extract produces benefits that may be enhanced by simultaneous intake of ethanol.

Vinson et al. (2002) studied the effect of a grape seed extract, with or without niacin-bound chromium, in a hamster model of atherosclerosis. The tested grape seed extract was a commercial product known as ActiVin, comprising 76% oligomeric proanthocyanidins and 3% monomeric bioflavonoids. Male weanling Syrian golden hamsters were acclimated for 4 weeks, then divided into groups of 7-9 hamsters/group and housed 3-4 animals/cage with *ad libitum* access to feed and water; the feed was a high-fat high-cholesterol chow that provided a hypercholesterolemic diet. The control group (n = 8) received the unsupplemented chow, 2 test groups (n = 9/group) received chow supplemented with 50 or 100 ppm grape seed extract, and 1 test group (n = 7) received chow supplemented with 50 ppm grape seed extract and 2.2 ppm chromium for 10 weeks.

The addition of grape seed extract to the diet at either 50 or 100 ppm significantly reduced the formation of pre-atherosclerotic plaque, addition of grape seed extract at 100 ppm

significantly reduced TG, and the combination of grape seed extract and chromium significantly reduced TC. The authors suggested that "some of the heart benefits of red wine can be obtained by consumption of nonalcoholic supplements such as [grape seed extract]." There were no differences in feed consumption or weight gain in any of the groups and no adverse effects were reported.

Male golden Syrian hamsters fed an atherosclerotic diet were used in a study by Auger et al. (2004) comparing the effects of 3 commercially available grape extracts. These included exGrape® Total grape pomace extract (the subject of the current GRAS notice), exGrape® seed extract by the same company, and an unidentified competing grape seed extract. Thirty-two hamsters weighing 60-80 g were assigned to 4 groups (n = 8/group) and group-housed with free access to feed (high-cholesterol chow as described above) and water for 12 weeks, with bodyweight and feed disappearance measured daily. Hamsters in the experimental groups were force-fed one of the 3 test extracts in water at concentrations providing 14.3 mg phenols/kg bw/day while the control hamsters received pure water.

After 12 weeks the animals were fasted for 18 hours, anesthetized, and subjected to a blood draw. After sacrifice, aortas were excised and dissected for examination of epithelial tissue. Blood was analyzed for TC, apolipoprotein A-1 and B, and plasma TAC. Neither feed intakes nor final body weights were significantly different across the 4 groups and no differences were seen in plasma Apo A1 or Apo B or in TAC. However, all 3 extracts significantly reduced TC and atherosclerotic streaks in the aortas and the authors concluded that "phenolic extracts from grape seeds and marc are beneficial in inhibiting atherosclerosis by indirect mechanism(s)." No adverse effects were noted from 12 weeks' administration of any of the phenolic extracts.

4.3.4. Guinea Pigs

Using ovariectomized guinea pigs as a model for menopausal women, Zern et al. (2003) studied the effect of a lyophilized grape preparation on markers of cardiovascular disease. Grape powder was prepared in the laboratory from fresh red, green, and blue-black California grapes; the total phenol content was 580 mg/100 g, including 410 mg flavans and 77 mg anthocyanins. Twenty-three female ovariectomized guinea pigs weighing 250-300 g were randomly assigned to receive a high-cholesterol diet supplemented with 10% grape powder (n = 11) for 12 weeks or the same diet without the grape powder (n = 12). The animals were housed 2/cage with free access to feed and water. At the end of the in-life phase the guinea pigs were exsanguinated and their livers and aortas excised. Blood was analyzed for TC, HDL, TG, VLDL Apo-B, lecithin cholesterol acyltransferase (LCAT), cholesterol ester transfer protein (CETP), and LDL-grain-size determination; liver lipids for TC, free cholesterol, TG, and acyl-CoA cholesterol acyltransferase (ACAT) activity; and aortas for TC.

Although feed consumption was not assessed, there were no differences in bodyweights between the groups and the authors therefore questionably assumed that feed intakes were approximately equal. No differences were observed in plasma TC, LDL, HDL, LCAT, CETP, or LDL particle size, nor in hepatic TC or TG, but diet supplementation with grape polyphenols significantly lowered plasma TG and VLDL as well as hepatic ACAT activity and aortic TC. The authors concluded that "even in the absence of estrogen, polyphenols exert their protecitve effects [against heart disease]." The effects seen were regarded as beneficial and no adverse effects were reported.

4.3.5. Conclusions from Animal Studies

In addition to the animals exposed to grape seed and grape skin extracts in formal studies of oral toxicity, a variety of animal species—mice, rats, hamsters, and guinea pigs—has ingested such extracts with no indications of harm in 18 published studies. While measured endpoints that might have revealed adverse effects were sparse in some research that focused on specific parameters such as blood lipids or oxidative stress, other studies (such as Bagchi et al. 2001, Al-Awwadi et al. 2005, Cetin et al. 2008, and Zern et al. 2003) included hematological and biochemical measures more often seen only in toxicity studies. The absence of any evidence of toxicity in these studies adds significantly to the robustness of the conclusion of safety resulting from formal studies of oral toxicity of grape extracts.

Table 15. Safety-Related Findings In Animal Studies.

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Al-Awwadi et al. (2005)	Study the capacity of 3 different grape seed extracts to prevent hypertension, cardiac hypertrophy, increased production of reactive oxygen species, and increased expression of cardiac NADPH oxidase in diabetes mellitus	One group received standard chow as a control, while the other 4 groups were given feed enriched with 60% fructose to induce insulin resistance. Three of the high-fructose groups received daily gavage of 10 ml grape seed extract/kg bw, one extract enriched in anthocyanins, one enriched in procyanidins and galloylated procyanidins, and one rich in catechin oligomers.	45 Sprague-Dawley rats weighing 185-220 g	All treatments provided 2421 mg polyphenols/ kg bw/day	6 weeks	There was no mortality and no significant differences in body weight, AST, ALT, BUN, blood glucose, and insulin. The rats receiving the high-fructose diet had significantly elevated TG and insulin resistance compared to controls, which were fully reversed by the grape seed extract high in procyanidins and partially reversed by the other extracts. None of the extracts reduced the elevations in phospholipids or LDL induced by the high-fructose diet, but all of them significantly lowered the induced hypertension, cardiac hypertrophy, and increase superoxide-anion production. All of the effects of the 3 grape seed extracts were regarded as beneficial, with no reported adverse effects.
Auger et al. (2002)	Study the effect of red-grape phenolics on plasma lipids and early aortic atherosclerosis	Hamsters were divided into 4 groups receiving phenolic extract along with either pure water (Group 1) or water with ethanol (Group 2), while Groups 3 and 4 received only pure water or water with ethanol with no phenolic extract.	32 male golden Syrian hamsters weighing 60-80 g	30.4 mg phenolic extract/kg bw/day (total phenols = 471 mg/g)	8 weeks	Feed consumption differed little among the groups (although it was statistically significantly higher by Group-1 hamsters than the other groups) and there were no significant differences in final body weights. Hamsters that received phenolic extract had significantly reduced TC, heightened TAC, and reduced aortic fatty streaks, but TG, glutathione peroxidase activity and Apo-A1 did not differ among groups. Phenolic extract had no effect on HDL, but it was reduced by ethanol. No adverse effects associated with administration of phenolic extract were noted. The authors concluded that phenolic extract produces benefits that may be enhanced by simultaneous intake of ethanol.

Table 15. Safety-Related Findings In Animal Studies.

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Auger et al. (2004)	Compare the effects of 3 grape extracts on animals fed an atherosclerotic diet: exGrape® Total, exGrape® seed extract by the same company, and unidentified grape seed extract	Hamsters in the experimental groups were force-fed an atherosclerotic diet and one of the 3 test extracts in water while the control hamsters received pure water.	32 male golden Syrian hamsters weighing 60-80 g	14.3 mg phenols/kg bw/day	12 weeks	Neither feed intakes nor final body weights were significantly different across the 4 groups and no differences were seen in plasma Apo A1 or Apo B or in TAC. However, all 3 extracts significantly reduced TC and atherosclerotic streaks in the aortas. No adverse effects were noted from 12 weeks' administration of any of the phenolic extracts.
Bagchi et al. (2001)	Study the ability of grape seed extract to protect against drug- and chemical-induced multiorgan toxicity caused by any of 6 agents	2x2 factorial designs with groups receiving saline or grape seed extract x toxic agent or no toxic agent. Mice received gavage prior to toxic-agent exposure: 7 days prior to cadmium chloride or acetamino-phen, 8 days prior to dimethyl-nitrosamine or *O*-ethyl-S,S-dipropyl phosphorodithioate, 9 days prior to doxo-rubicin, 10 days prior to amiodarone	3-month-old male ICR (CD-1) mice weighing 30-40 g	100 mg/kg bw/ day grape seed extract with 74% oligo-meric proan-thocyanidins (54% di-mers; 13% trimers, 7% tetramers)	See study design	Measured endpoints were serum ALT, BUN, and creatine kinase activity; counts of normal, apoptotic, and necrotic cells in target tissues; and DNA fragmentation in target tissues. No effects of ingestion of grape seed extract alone were observed in any endpoint in any of the experiments. The 6 agents inoculated alone consistently produced the expected toxic effects, which were significantly reduced or abolished by pretreatment with grape seed extract.

Table 15. Safety-Related Findings In Animal Studies.

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Cai et al. (2010)	Test the effect of an anthocyanin-rich grape seed extract on adenoma development in the *Apc*^{Min} mouse, a model for human familial adenomatous polyposis coli.	Mice were assigned to receive AIN-93G chow from week 4 to week 16 either with or without /) extract supplementation	*13-14* *Apc*^{Min} mice/ group	0.3% in feed; antho-cyanin 22%,	12 weeks	Supplementation with grape pomace extract significantly reduced adenoma development by about 50% with no reported adverse effects. In a pharmacokinetic side experiment, plasma, urine, and intestinal mucosa of mice which had received the extract were analyzed for anthocyanins, which were identified in urine and mucosa but not plasma. The authors regarded their findings as supportive of a role for anthocyanin-rich grape pomace extract in chemopreventive intervention.
Cetin et al. (2008)	Study the protective effect of grape seed extract on radiation-induced oxidative stress in the liver.	Rats were assigned to 4 groups. 2 groups received grape seed extract; rats in the other 2 groups received water. On day 7, one of the groups of rats receiving each treatment was exposed to 8 Gy whole-body irradiation	48 male Wistar rats weighing 240-320 g	100 mg grape seed extract/kg bw/day	11 days	Among the rats that did not receive radiation, there was no difference between those ingesting grape seed extract and the controls in white and red blood cell counts, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet count, or lymphocyte count. Radiation significantly depressed white and red blood cell counts, hematocrit, and lymphocyte count and increased the mean cell hemoglobin and mean cell hemoglobin concentration, but the treatment and control groups receiving radiation did not differ from each other. Similarly, although radiation significantly increased malondialdehyde and decreased superoxide dismutase and catalase, and ingestion of grape seed extract provided a significant protective effect, there was no difference between the test and control groups not receiving radiation. No adverse effects were reported due to ingestion of 100 mg grape seed extract/kg bw/day.

Table 15. Safety-Related Findings In Animal Studies.

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Dal-Ros et al. (2012)	Assess the ability of red-wine polyphenols to mitigate aging-related endothelial dysfunction in the mesenteric artery caused by oxidative stress	Rats consumed drinking water containing red wine polyphenols, ethanol, or apocynin; mesenteric artery rings were excised for measurement of tension and oxidative stress	15 51-week-old male Wistar rats	100 mg red wine polyphenols/ kg bw/day	4 weeks	Improvement was seen in age-related endothelial dysfunction of the mesenteric artery and oxidative stress. The authors concluded that "intake of red wine polyphenols, besides retarding the development of an age-related endothelial dysfunction, also improves an established aging-related endothelial dysfunction in the middle-aged rat." No adverse effects were reported from the treatment.
El-Adawi et al. (2006)	Investigate the ability of grape seed extract to prevent the development of or reduce already-developed hypercholesterolemia	Rats were fed normal rat chow or a high-cholesterol diet with or without grape seed extract	48 35-day-old male Wistar rats weighing 100-120 g	0.3% dietary concentration of grape seed extract (598.8 mg polyphenols/g)	8 weeks or longer	All of the rats receiving the unsupplemented high-cholesterol diet developed hypercholesterolemia within 8 weeks, with TC concentrations double those of the control group and LDL levels 5 times higher. Addition of grape seed extract did not prevent the development of hypercholesterolemia, but TC and LDL were lower than in the unsupplemented rats. Grape seed extract also reduced both TC and LDL in the rats that had already developed the disease. No adverse effects were reported on rats consuming 0.3% grape seed extract.
Lionetto et al. (2011)	Evaluate the effect of red grape skin extract on serum antioxidant capacity	1. Rats divided into 2 groups received oral gavage providing 0 or 0.6 mg grape skin extract/kg bw/day 2. Rats assigned to 4 groups in a 2x2 factorial design received 0 or 0.6 mg grape skin extract/ kg bw/day, x sc injection of CCl_4 to produce oxidative stress or not	48 male Wistar rats weighing 200-250 g	0 or 0.6 mg grape skin extract/ kg bw/day; 50% malvidin 3-*O*-glucoside, 10% petunidin 3-*O*-glucoside, 8% delphinidin 3-*O*-glucoside, and 5% cyanidin 3-*O*-rutinoside	10 days	In the first experiment, the daily gavage with grape skin extract produced a statistically significant 50% increase in antioxidant capacity. The second experiment showed that ingestion of the grape skin extract was capable of completely offsetting the prooxidant effect of carbon tetrachloride administration. No adverse effects were reported. Additionally, in an *in vitro* study with excised segments of rat jejunal mucosa, the authors showed that direct contact of the grape skin extract with the luminal surface of the mucosa for 3 hours resulted in no effect on its permeability or integrity.

Table 15. Safety-Related Findings In Animal Studies.

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Pataki et al. (2002)	Study the effects of red grape seed proanthocyanidins on the recovery of postischemic function	Rats were divided into 3 groups and given daily gavage doses of 0, 50, or 100 mg extract/kg bw After sacrifice the rats' hearts were excised, subjected to 30 minutes of ischemia, and given 2 hours of reperfusion	36 17-19-week-old Sprague-Dawley rats weighing 320-340 g	0, 50, or 100 mg extract/ kg bw (54% dimeric pro-anthocyanidins, 13% trimers, 7% tetramers	3 weeks	Ingestion of grape seed extract resulted in a significant dose-dependent reduction in the production of oxygen free radicals. The treatment had no effect on feed intake or body weight, and no adverse effects were reported.
Santa-Maria et al. (2011)	Assess the effect of grape seed extract on the intracellular accumulation of hyperphosphory-lated tau protein in the spinal cords of mice expressing a human tau protein containing the P301L mutation	Test mice received grape seed extract in their drinking water while control mice received pure water	16 9-month-old male JNPL3 transgenic mice ex-pressing a human tau protein containing the P301L mutation	150 mg grape seed extract/kg bw/day	6 months	The authors stated that "Long-time [grape seed extract] treatment for 6 months was well tolerated as reflected by normal grooming behavior and steady body weight values." The treatment significantly reduced levels of hyperphosphorylated and conformationally modified tau in spinal-cord tissue and significantly improved neuromuscular function, and the authors suggested that such intervention may be of value in human tauopathies.

Table 15. Safety-Related Findings In Animal Studies.

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Tebib et al. (1994)	Study the effect of consumption of grape seed extract on lipids and lipases in male Sprague-Dawley rats fed hyper-cholesterolemic diets	Rats were housed in metabolic cages and divided into 4 groups. Controls received a diet of standard rat chow while a second group received chow with 38% saturated fatty acids and 1% cholesterol. The remaining groups were fed the hypercholesterolemic diet supplemented by procyanidin monomers or polymers	48 male Sprague-Dawley rats weighing about 190 g	Procyanidin monomers or polymers at a 2% dietary concentration	9 weeks	Rats receiving procyanidin polymers had the lowest levels of TC, LDL- and VLDL-cholesterol, and the highest levels of HDL-cholesterol, all significantly different from the rats receiving the hypercholesterolemic diet without grape seed extract; the rats with monomer supplementation were between the other groups. There was no difference between groups in body protein, but body fat was significantly lower in the polymer-supplemented group, as was lipoprotein lipase activity. The authors concluded that "dietary polymeric grape seed tannins reduce plasma total cholesterol, triacylglycerol, and LDL cholesterol concentrations in high cholesterol fed rats," with no adverse effects reported from consuming monomers or polymers at 2% dietary concentration for 9 weeks.
Tebib et al. (1996)	Study the effect of grape seed extract on colonic bacterial enzymes and cecal fermentation	Rats were housed in metabolic cages and divided into 3 groups to receive standard rat chow or rat chow supplemented with procyanidin monomers or polymers.	18 male Sprague-Dawley rats weighing about 145 g	0.71% dietary concentration of procyanidin monomers or polymers	12 weeks	There were no differences in feed intake, but rats fed chow with procyanidin polymers gained less weight than rats in the other 2 groups. Rats receiving polymers had thinner cecal walls, higher concentrations of volatile fatty acids, and lower pH cecal contents. The polymer group had higher nitrogen content of colonic contents. There was no difference between groups in overall level of colonic bacterial enzymes, but activities of β-glucosidase and mucinase were significantly lower in rats that had ingested procyanidin polymers. The authors concluded that "polymeric grape seed tannins intake lead to a stimulation of fermentative activities [seen in increased concentrations of volatile fatty acids in the cecum] without an increase of deleterious enzymic activities."

Table 15. Safety-Related Findings In Animal Studies.

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Vinson et al. (2002)	Study the effect of a grape seed extract, with or without niacin-bound chromium, in a hamster model of atherosclerosis	Hamsters were given a hypercholesterolemic diet; 2 test groups got chow with 50 or 100 ppm grape seed extract; 1 test group got chow with 50 ppm grape seed extract and 2.2 ppm Cr; controls got unsupplemented chow	33 male weanling Syrian golden hamsters	50 or 100 ppm grape seed extract with 76% oligomeric proantho-cyanidins & 3% mono-meric bio-flavonoids	10 weeks	The addition of grape seed extract to the diet at either 50 or 100 ppm significantly reduced the formation of pre-atherosclerotic plaque, addition of grape seed extract at 100 ppm significantly reduced TG, and the combination of grape seed extract and chromium significantly reduced TC. The authors suggested that "some of the heart benefits of red wine can be obtained by consumption of nonalcoholic supplements such as [grape seed extract]." There were no differences in feed consumption or weight gain in any of the groups and no adverse effects were reported.
Wang et al. (2008)	Study the effect of grape seed extract on aggregation of amyloid β-protein into high-molecular-weight Aβ oligomers, a precursor to Alz-heimer's disease	Mice received either pure drinking water or water supplemented with grape seed extract	Adult female Tg2576 AD transgenic mice	200 mg grape seed extract/kg bw/day (contains 8% monomers, 75% oligo-mers, 17% polymers	5 months	Mice receiving grape seed extract performed significantly better than controls in the water-maze test and showed significantly less oligomerization of Aβ peptides into high-molecular-weight species and significant reduction of total and soluble Aβ levels, but no change in amyloid precursor protein or in α-, β-, or γ-secretase activity. The authors reported that, "[grape seed extract] treatment delivered in the drinking water for 5 months did not result in detectable adverse effects, including changes in body weight or water consumption. Normal liver functions were observed in both [grape seed extract]-treated and water-treated control groups, as reflected by normal serum levels of aspartate aminotransferase and alanine aminotransferase."

Table 15. Safety-Related Findings In Animal Studies.

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Wang et al. (2010)	Study the ability of grape seed extract to modulate the onset and progression of Huntington's disease	Mice were randomly assigned to receive pure water or grape seed extract and were then mated with wild-type male mice; pups received the same treatment as their parents	R6/2 ovarian-transplant female mice and their pups	100 mg/kg bw/day of grape seed extract with 8% mono-mers, 75% oligomers, and 17% polymers	11 weeks	No difference in performance was seen at 6 weeks, but at 9 and 11 weeks the mice receiving grape seed extract had significantly better motor coordination than the controls. Grape seed extract also significantly extended the median lifespan of the rats from 90.5 days to 98 days. The authors noted at the treatment with grape seed extract was "well tolerated," with no adverse effects apparent.
Wen et al. (2008)	Study the ability of a grape seed extract to inhibit tumor formation in mice	The mice were gavaged with water containing 0 or 50 mg/kg bw grape seed extract, followed by an injection of breast-cancer cells; the mice continued to be gavaged daily with water or grape seed extract	11 6-8-week-old SCID mice	50 mg/kg bw/day grape seed extract containing ≥85% pro-cyanidins	11 weeks	Treatment with grape seed extract significantly inhibited tumor growth and the "treatment seemed to have no obvious toxicity and showed no detectable effect on body weight and behavior of mice."
Zern et al. (2003)	Study the effect of a lyophilized grape preparation on markers of cardiovascular disease using ovariectomized guinea pigs as a model for menopausal women	Guinea pigs were randomly assigned to receive a high-cholesterol diet supplemented with 10% grape powder or the same diet without the grape powder	23 female ovariec-tomized guinea pigs weighing 250-300 g	10% dietary concentra-tion with phenolic content of 580 mg/100 g, including 410 mg flavans and 77 mg an-thocyanins	12 weeks	Feed consumption was not assessed, but there were no differences in bodyweights in the groups and the authors assumed that feed intakes were approximately equal. No differences were observed in plasma TC, LDL, HDL, LCAT, CETP, or LDL particle size, nor in hepatic TC or TG, but diet supplementation with grape polyphenols significantly lowered plasma TG and VLDL as well as hepatic ACAT activity and aortic TC. These differences were regarded as beneficial and no adverse effects were reported.

4.4. Human Studies

The studies discussed below are summarized in Table 16 at the end of the section.

Nigdikar et al. (1998) extracted polyphenolic compounds from *Cabernet sauvignon* grapes using an absorbent resin column and ethanol elution and spray-dried the eluate to produce a powder with a polyphenol concentration of 450 mg/g, primarily procyanidins and anthocyanin glycosides. In an open-label study, 30 apparently healthy, nonsmoking, nonobese, normolipemic men aged 35-65 years were randomly assigned to 5 groups (n = 6 men/group) to receive test polyphenol sources along with their usual diet for 2 weeks: (1) 275 ml red wine/day, (2) 275 ml white wine/day, (3) 275 ml white wine + 1 g polyphenol powder/day, (4) 1 g polyphenol powder/day, or (5) no wine or polyphenol powder, but 40 ml ethanol/day in lemonade. Blood samples were taken at baseline and after the 2-week test period and analyzed for plasma polyphenol and lipid peroxide concentrations, and thiobarbituric acid–reactive substances (TBARS)

Plasma polyphenols increased and lipid peroxides and TBARS decreased significantly among men consuming either red wine or polyphenol powder, but not in the other groups. No adverse effects were noted from any of the treatments.

In a randomized, single-blinded, placebo-controlled, crossover study (Nutall et al. 1998), 20 apparently healthy non-smoking students (11 males, 9 females) aged 19-31 years (mean age = 23±9.1 years) and weighing 54-84 kg (mean weight = 67.8±9.3 kg) provided resting blood samples, then consumed breakfast with 2 capsules providing either 300 mg Leucoselect™-phytosome® powder[1] or placebo. Further blood samples were taken after 30, 60, 90, 120, 180, and 240 minutes. The students continued to take the active treatment or placebo capsules for 4 days and on day 5 repeated the blood sampling performed on day 1. After a 2-week washout, students followed the same procedure with the alternative treatment. Blood samples were analyzed for TAC and vitamins C and E.

TAC was significantly increased within 30 minutes of consumption of the polyphenolic powder and continued to increase up to 60 minutes, remaining elevated over 3 hours post-dose. The same pattern was seen on day 5. Neither vitamin C nor vitamin E concentrations were significantly altered. No adverse effects were reported.

Natella et al. (2002) enrolled 8 apparently healthy men aged 25-40 years in a randomized, unblinded, uncontrolled cross-over study of postprandial hyperlipemia. Preceding 2 meals separated by 2 weeks, the men either did or did not consume 3 capsules providing 300 mg Leucoselect™ grape seed extract containing about 15% catechin and epicatechin; 80% epicatechin 3-O-gallate, dimers, trimers, tetramers, and their gallates; and 5% oligomers and

[1] The only information provided regarding the test article is that the dose administered contained "the equivalent of 300 mg of grape procyanidins."

their gallates. Blood draws were taken before the meal and 1 and 3 hours after it and analyzed for TAC, ascorbic acid, TC, TG, uric acid, lipid hydroperoxides in chylomicron-VLDL fractions

As expected, TC and TG levels increased postprandially, and consumption of grape seed extract had no effect. Lipid hydroperoxides also increased and grape seed extract significantly reduced this rise. TAC increased only when the meal was preceded by ingestion of grape seed extract. All of the effects of the extract were regarded as beneficial and no adverse effects were reported.

Kalfin et al. (2002) enrolled systemic sclerosis patients in a prospective, randomized, double-blind trial; oddly, the number, sex, age, and weight of the patients were not reported. For 30 days, one group of patients ingested 100 mg/kg bw/day of Activin, a commercially available grape seed extract containing about 75-80% oligomeric proanthocyanidins and 3-5% monomeric proanthocyanidins, while another group of patients did not; whether they ingested a placebo was not reported. Blood was drawn at baseline and at the end of the 30-day trial and analyzed for the adhesion molecules VCAM-1, ICAM-1, E-selectin, and P-selectin, and for malondialdehyde, a marker for oxidative stress. Blood was also taken for similar analyses from apparently healthy adults.

The sclerosis patients exhibited elevated levels of VCAM-1, ICAM-1, and E-selectin as compared with healthy adults, but these levels as well as those of malondialdehyde were significantly lowered by 30 days ingestion of grape seed extract. These changes were beneficial in reducing the inflammatory response and oxidative stress of systemic sclerosis. The authors reported that "No side effects were detected during the treatment."

In an uncontrolled open-label study, Piper et al. (2005) studied the effects of ingestion of black grape extract and perilla oil in 100 atherosclerosis and essential arterial hypertension patients, 60 men and 40 women, aged 40-72 (mean age = 57±6 years) and weighing 59-137 kg (mean weight = 84±14 kg). Most of the patients were overweight, 42% had manifest type 2 diabetes mellitus, and 24% met the WHO definition for metabolic syndrome. All patients consumed capsules containing the test articles 3 times/day, providing a daily total of 1.6 g of α-linolenic acid and 600 mg black grape extract which contained ≥ 270 mg polyphenols and ≥ 60 µg resveratrol. Patients remained on the regimen for 12 weeks. Physical examinations and blood draws were performed at baseline and at the end of the in-life phase; measures included body weight and BMI, systolic and diastolic blood pressure, heart rate, plasma TC, LDL, HDL, TG, and blood glucose, and patients completed quality-of-life questionnaires.

No significant change was seen in body weight or BMI, but heart rate and both systolic and diastolic blood pressures were significantly lower at the end of the intervention period than at the beginning (mean heart rate 74.6 and 72.3 beats/minute; mean systolic pressure—151.0 and 137.8 mmHg; mean diastolic pressure—93.1 and 83.3 mmHg). Blood glucose levels decreased significantly in both diabetic and non-diabetic patients, as did TC, LDL, and TG concentrations, while HDL levels increased significantly. The success of the dietary intervention was generally rated as good or very good, as was patients' tolerance for the intervention. The authors noted that, "There were no reports of any undesired side effects."

Sivaprakasapillaia et al. (2009) studied the effect of grape seed extract on blood pressure of patients with metabolic syndrome in a prospective, randomized, double-blind, placebo-controlled trial. The test article was MegaNatural™ BP, with a phenol content of about 94%,

composed primarily of catechin units with an average polymerization of 2.4. Twenty-seven metabolic-syndrome patients, 11 males and 16 females, aged 25-80 years (mean age = 46±3 years) and with mean BMI = 36±2.4 were enrolled and randomized into 3 groups to receive capsules containing a placebo or either 150 or 300 mg grape seed extract/day for 4 weeks during which their blood pressure was recorded at 1-hour intervals. Fasting blood samples were collected at baseline and termination and analyzed for hemoglobin, total and differential white cell count, serum lipids, glucose, and insulin. Five patients in the placebo and high-dose groups provided additional blood samples before and 90 minutes after ingestion of a capsule for measurement of plasma catechin.

The catechin analysis showed significantly increased plasma levels of catechin after consumption of a capsule containing grape seed extract. Both systolic and diastolic blood pressures decreased significantly in the patients receiving grape seed extract as compared to the controls, but no dose-dependence was evident. (Changes in systolic pressures were -1, -12, and -11 mmHg in the control, low-dose, and high-dose groups respectively, while changes in diastolic pressures were -2, -7, and -8 mmHg, respectively.) No changes were seen in heart rates or in TC, HDL, LDL, or oxidized-LDL levels, nor in the complete blood counts or in glucose, sodium, potassium bicarbonate, creatinine, or blood urea nitrogen. No adverse effects were reported, and the authors suggested that grape seed extract could be used "in a lifestyle modification program for patients with the metabolic syndrome."

In a prospective, randomized, double-blind, placebo-controlled crossover study, 35 apparently healthy men aged 18-45 years (mean age = 31.4±9.0 years) with mean bodyweight = 78.1±10.5 kg ingested 6 capsules daily providing 3000 mg wine grape extract, grape seed extract, or microcrystalline cellulose placebo for 2 weeks, separated by 1-week washout periods (van Mierlo et al. 2010). Both extracts contained 27% polyphenols in gallic acid equivalents; the wine grape extract was derived from red grape juice while the grape seed extract was composed of Leucoselect, a commercially available product. Body weight, blood pressure, flow-mediated arterial dilation, pulse rate, platelet function, and blood lipids were measured twice at baseline and at the end of each intervention period immediately following a low-fat breakfast and after a high-fat lunch.

All enrolled individuals completed the study with an estimated compliance rate of 86% (based on test product disappearance). Body weight, flow-mediated dilation, blood pressure, pulse rate, and platelet function were not affected by the treatments. Compared with the placebo group, those ingesting wine grape extract, but not grape seed extract, had significantly lower TC and TG after the high-fat lunch (-0.20 and -0.19 mmol/L, respectively) and non-significantly lower TC and TG after the low-fat breakfast; there were no differences in HDL and LDL concentrations. A total of 39 adverse events were noted, all of which "were determined to be unrelated to any of the test products."

Hassellund et al. (2012) studied the effects of anthocyanins on blood lipids, oxidative stress, and inflammation on prehypertensive men in a prospective, randomized, double-blind, placebo-controlled, crossover trial. The test article was Medox capsules containing 80 mg anthocyanins (primarily cyanidin 3-O-β-glucosides and delphinidin 3-o-β-glucosides) based on analyses of each batch of capsules produced. Thirty-one men aged 35-51 years (mean age = 41±3 years) with either systolic blood pressure > 140 or diastolic pressure > 90 were enrolled and randomized to receive 4 capsules each morning and evening for 4 weeks; each capsule contained

either anthocyanins or maltodextrin. Following a 4-week washout, each participant consumed 4 capsules per day of the other regimen. The men visited the clinic every 4 weeks and gave blood samples for analysis of plasma phenolic acids, TC, HDL, LDL, TG, Lp(a), glucose, HbA1c, albumin, creatinine, insulin, homocysteine, inflammatory parameters (C-reactive protein, TNFα, IL-6, IL-4, MCP-1, CD40L, ICAM-1, VCAM-1, P-selectin, Von Willebrand factor, L-arginine, asymmetric dimethylarginine, and NOx), markers of oxidative stress (ferric-reducing/antioxidant power and reactive oxygen metabolites test), hematological markers, and measures of liver and kidney function.

Twenty-seven of the 31 men completed the study. No adverse reactions were reported during the placebo periods although 3 patients withdrew for unrelated reasons; during the anthocyanin period one patient withdrew due to diarrhea, 3 patients reported minor headaches, and 1 patient reported nausea. It was not determined whether these adverse effects could be related to treatment. In comparison to placebo, anthocyanin ingestion resulted in significantly higher levels of plasma phenolic acids, HDL, glucose, and the Von Willebrand marker of inflammation (phenolic acids—1.34 and 1.82 mmol/L; HDL—1.18 and 1.24 mmol/L; glucose—5.08 and 5.22 mmol/L; and von Willebrand factor—74 and 79%); no differences were seen in the hematological and biochemical markers white blood cell and platelet counts, fibrinogen, uric acid, urea, creatinine, AST, or ALT. The authors concluded that, while "purified anthocyanins may increase HDL-C in prehypertensive and non-dyslipidemic participants," they "do not beneficially affect markers of inflammation, endothelial dysfunction, or oxidate stress in the short-term"

4.4.1. Conclusions from Human Studies

Eight published studies in which either healthy adults or patients diagnosed with metabolic syndrome, atherosclerosis, essential arterial hypertension, or systemic sclerosis ingested grape seed extract at doses up to 100 mg/kg bw/day for up to 12 weeks provide further evidence that such ingestion has no harmful effects on humans. The studies provided some evidence of benefits in reduced blood levels of peroxides, increased antioxidant capacity and reduced blood pressure, and certain measures of blood lipids, but—more importantly—there was an absence of any observed adverse effects clearly attributable to treatment.

Table 16. Safety-Related Findings in Human Studies.

Citation	Study Design	Subjects	Dose	Duration	Results
Hassellund et al. (2012)	Prospective, randomized, double-blind, placebo-controlled, crossover trial	31 men aged 35-51 years (mean age = 41 years) with systolic pressure > 140 or diastolic pressure > 90	640 mg/day of purified anthocyanins (primarily cyanidin 3-O-β-glucosides and delphinidin 3-o-β-glucosides)	4 weeks	During the anthocyanin period 1 patient withdrew due to diarrhea, 3 patients reported minor headaches, and 1 patient reported nausea. It was not determined whether these adverse effects could be related to treatment. Anthocyanins resulted in higher levels of plasma phenolic acids, HDL, glucose, and the Von Willebrand marker of inflammation; no differences were seen in white blood cell and platelet counts, fibrinogen, uric acid, urea, creatinine, AST, or ALT. The authors concluded that, while "purified anthocyanins may increase HDL-C in prehypertensive and non-dyslipidemic participants," they "do not beneficially affect markers of inflammation, endothelial dysfunction, or oxidate stress in the short-term"
Kalfin et al. (2002)	Prospective, randomized, double-blind trial	Systemic sclerosis patients (n not reported)	100 mg/kg bw/day of grape seed extract containing 75-80% oligomeric proanthocyanidins and 3-5% monomeric proanthocyanidins	30 days	The sclerosis patients exhibited elevated levels of VCAM-1, ICAM-1, and E-selectin as compared with healthy adults, but these levels as well as those of malondialdehyde were significantly lowered by 30 days ingestion of grape seed extract. These changes were beneficial in reducing the inflammatory response and oxidative stress of systemic sclerosis. The authors reported that "No side effects were detected during the treatment."
Natella et al. (2002)	Prospective, randomized, unblinded, uncontrolled cross-over	8 apparently healthy men aged 25-40 years	300 mg grape seed extract containing about 15% catechin and epicatechin; 80% epicatechin 3-O-gallate, dimers, trimers, tetramers, and their gallates	2 meals	TC and TG levels increased postprandially and consumption of grape seed extract had no effect. Lipid hydroperoxides also increased and grape seed extract significantly reduced this rise. TAC increased only when the meal was preceded by ingestion of grape seed extract. All of the effects of the extract were regarded as beneficial and no adverse effects were reported.
Nigdikar et al. (1998)	Open-label	30 apparently healthy men aged 35-65	(1) 275 ml red wine/day, (2) 275 ml white wine/day, (3) 275 ml white wine + 1 g polyphenol powder/day, (4) 1 g polyphenol powder/day	2 weeks	Plasma polyphenols increased and lipid peroxides and TBARS decreased significantly among men consuming either red wine or polyphenol powder, but not in the other groups. No adverse effects were noted from any of the treatments.

Table 16. Safety-Related Findings in Human Studies.

Citation	Study Design	Subjects	Dose	Duration	Results
Nutall et al. (1998)	Prospective, randomized, single-blinded, placebo-controlled, crossover study	20 healthy students, 11 males and 9 females, age 19-31 years	300 mg Leucoselect™-phytosome® powder (not further characterized)	5 days	TAC was significantly increased within 30 minutes of consumption of the polyphenolic powder and continued to increase up to 60 minutes, remaining elevated over 3 hours post-dose. The same pattern was seen on day 5. Neither vitamin C nor vitamin E concentrations were significantly altered. No adverse effects were reported.
Piper et al. (2005)	Uncontrolled open-label study	100 athero-sclerosis and essen-tial arterial hypertension patients, 60 men and 40 women, age 40-72 (mean age = 57)	600 mg/day of black grape extract which contained ≥ 270 mg polyphenols and ≥ 60 µg resveratrol	12 weeks	No significant change was seen in body weight or BMI, but heart rate and both systolic and diastolic blood pressures were significantly lower at the end of the intervention period than at the beginning. Blood glucose levels decreased significantly in both diabetic and non-diabetic patients, as did TC, LDL, and TG concentrations, while HDL levels increased significantly. The success of the dietary intervention was generally rated as good or very good, as was patients' tolerance for the intervention. The authors noted that, "There were no reports of any undesired side effects."
Sivaprakasa-pillaia et al. (2009)	Prospective, randomized, double-blind, placebo-controlled trial	27 patients with metabolic-syndrome, 11 males and 16 females, aged 25-80 years (mean age = 46)	150 or 300 mg grape seed extract/day (phenol content of 94% composed primarily of catechin units with a mean polymeriza-tion of 2.4	4 weeks	Significantly increased plasma levels of catechin were seen after consumption of capsules containing grape seed extract. Systolic and diastolic blood pressures decreased significantly in the patients receiving grape seed extract as compared to the controls, but no dose-dependence was evident. No changes were seen in heart rates or in TC, HDL, LDL, or oxidized-LDL levels, nor in the complete blood counts or in glucose, sodium, potassium bicarbonate, creatinine, or blood urea nitrogen. No adverse effects were reported, and the authors suggested that grape seed extract could be used "in a lifestyle modification program for patients with the metabolic syndrome."

Table 16. Safety-Related Findings in Human Studies.

Citation	Study Design	Subjects	Dose	Duration	Results
van Mierlo et al. (2010)	Prospective, randomized, double-blind, placebo-controlled crossover study	35 healthy men aged 18-45 years (mean age = 31.4 years)	3000 mg/day wine grape extract or grape seed extract, both containing 27% polyphenols	2 weeks	All enrolled individuals completed the study. Body weight, flow-mediated dilation, blood pressure, pulse rate, and platelet function were not affected by the treatments. Compared with the placebo group, those ingesting wine grape extract, but not grape seed extract, had significantly lower TC and TG after the high-fat lunch and non-significantly lower TC and TG after the low-fat breakfast; there were no differences in HDL and LDL concentrations. A total of 39 adverse events were noted, all of which "were determined to be unrelated to any of the test products."

5. SAFETY ASSESSMENT AND GRAS DETERMINATION

5.1. Introduction

This chapter presents an assessment that demonstrates that the intended use of exGrape® Total grape pomace extract is safe, and is GRAS, for direct addition to conventional foods. This GRAS determination is based on scientific procedures.

This safety assessment and GRAS determination entail two steps. In the first step, the safety of exGrape® Total grape pomace extract under its intended conditions of use is demonstrated. Safety is established by demonstrating that the likely intake of grape pomace extract under its intended conditions of use is within levels of intake that have been shown to be safe. In the second step, exGrape® Total grape pomace extract is determined to be GRAS by demonstrating that the safety of this substance under its intended conditions of use is generally recognized among qualified scientific experts and is based on publicly available and accepted information.

5.2. Previous FDA Opinions on the Safety of Grape Extracts

In a GRAS notice dated January 28, 2003, and filed by FDA on February 14, 2003, as GRN 000124, San Joaquin Valley Concentrates informed FDA that it had concluded that the addition of grape seed extract at levels of 0.01 to 0.08% (100 to 800 ppm) to beverages and beverage bases, breakfast cereals, fats and oils, frozen dairy desserts and mixes, grain products, milk (whole and skim), milk products, processed fruits and fruit juices is both safe and GRAS. The basis for these conclusions included pharmacokinetic studies; studies of oral, reproductive, and genetic toxicity; and clinical, epidemiological, and nutritional studies. In a letter dated August 1, 2003, FDA responded that "the agency has no questions at this time regarding San Joaquin's conclusion. "

As noted earlier, a GRAS notice was submitted by Polyphenolics, Inc., on Feburary 6, 2003, designated GRN 000125 by FDA. The notice informed FDA that Polyphenolics had determined that addition of grape seed extract and grape skin extract (referred to by FDA as grape pomace extract) to fruit juices, fruit-flavored beverages, fruit-flavored beverage mixes, and carbonated fruit-flavored beverages at a concentration of up to 210 ppm is both safe and GRAS. After reviewing the evidence cited by Polyphenolics, including pharmacokinetic studies, studies of oral and genetic toxicity, and nutritional and clinical studies, FDA concluded that "the agency has no questions at this time regarding Polyphenolics' conclusion " (letter from FDA dated August 18, 2003).

Since those two GRAS notices were submitted, a substantial amount of additional research on the safety of grape seed or pomace extracts has been published, including newer pharmacokinetic studies in animals and humans, additional studies of toxicity—especially genotoxicity—more than a dozen studies in various animal species, and several newer nutritional or clinical studies in both healthy and compromised humans, confirming the earlier conclusions of reasonable certainty of no harm from the ingestion of grape seed and pomace extracts under the conditions described in the GRAS notices.

5.3. Regulatory Framework

The regulatory framework for establishing whether the intended use of a substance is GRAS, in accordance with Section 201(s) of the Federal Food Drug and Cosmetic Act, is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under §170.30(c). This GRAS determination employs scientific procedures established under §170.30(b).

A scientific procedures GRAS determination requires the same quantity and quality of scientific evidence as is needed to obtain approval of the substance as a food additive. In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components:

1. Data and information relied upon to establish the scientific element of safety must be generally available; and
2. There must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific-procedures GRAS determination are applied below in an analysis of whether the addition of exGrape® Total grape pomace extract to food as a nutrient supplement is safe and is GRAS.

A scientific procedures GRAS determination requires that information about the substance establish that the intended uses of the substance are safe. The FDA has defined "safe" or "safety" for food additives under 21 CFR §170.3(i) as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use." This same regulation specifies that three factors must be considered in determining safety. These three factors are:

- The probable consumption of the substance and of any substance formed in or on food because of its use (i.e., the EDI);
- The cumulative effect of the substance in the diet, taking into account any chemically- or pharmacologically-related substance or substances in such diet; and
- Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

5.4. Safety of the Intended Use of exGrape® Total Grape Pomace Extract

5.4.1. EDI of Grape Pomace Extract

Based on data from the U.S. Department of Agriculture's 1994-96 Continuing Surveys of Food Intakes by Individuals and the 1998 Supplemental Children's Survey as cited in GRN125, the 90th percentile per-user intake of exGrape® Total grape pomace extract under its intended

conditions of use (which are the same as those of the MegaNatural™ products that were the subject of GRN125) is 130 mg/person/day, equivalent to 4 mg/kg bw/day. Further, because exGrape® Total grape pomace extract merely provides an alternative to the food formulator, no additional consumer exposure to grape pomace extract will result from the use of the exGrape® Total brand.

5.4.2. Animal And Human Research Establishing the Safety of Grape Pomace Extract

Four subchronic toxicity studies of grape seed and grape skin extracts in rats obtained NOAELs (always the highest doses tested) of 1410, 1586, 1780, and 1780 mg/kg bw/day in males and 1501, 1928, 2150, and 2150 mg/kg bw/day in females; a single subchronic study of grape skin extract in beagle dogs produced as a NOAEL the highest tested dose of approximately 2000 mg/kg bw/day in both sexes.

The single study of reproductive toxicity, in which grape seed extract was mixed into the diets of F_0-generation rats for 3 weeks before mating and through lactation, to the F_1 pups for 13 weeks before mating and through lactation, and to the F_2 generation pups for 21 days, further supports the safety of repeated ingestion of grape seed extracts. No adverse effects were seen on any endpoint, and the NOAEL for both reproductive effects and subchronic oral toxicity was the highest level tested, equivalent to 4000 mg grape seed extract/kg bw/day.

Based on these findings, a conservative estimate of a NOAEL for repeated-dose toxicity would be the highest tested dose in male rats, 1780 mg/kg bw/day. Application of a 100-fold uncertainty factor would produce an allowable daily intake (ADI) of 17.8 mg/kg bw/day, more than 4 times the EDI of grape seed extract from the intended use of exGrape® Total grape pomace extract.

The absence of consistent findings of genotoxicity in multiple assays of a variety of grape extracts and their phenolic components, as well as the absence of treatment-related adverse effects in numerous animal and human studies, lend further support to the conclusion that ingestion of grape seed or grape pomace extracts at levels as high as 17.8 mg/kg bw/day is safe.

5.5. General Recognition of the Safety of exGrape® Total Grape Pomace Extract

The intended addition of exGrape® Total grape pomace extract to food has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was established by first establishing the identity and purity of the material, estimating potential human exposure to grape pomace extract from its intended use, and demonstrating that this level of exposure is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the intended use of exGrape® Total grape pomace extract has been made through the deliberations of an Expert Panel consisting of Joseph F. Borzelleca, Ph.D., Walter H. Glinsmann, M.D., and Robert J. Nicolosi, Ph.D., who reached the following conclusion:

Conclusion

We, the undersigned expert panel members, are qualified by scientific education and experience to evaluate the safety of ingredients intended to be added to foods. We have independently and collectively critically reviewed and evaluated the publicly available information summarized in this document, including the potential human intake resulting from the intended uses of exGrape® Total grape pomace extract, as well as other information available to us, and have unanimously concluded that exGrape® Total grape pomace extract has been sufficiently characterized to ensure that it is a food-grade product and that no toxicity concerns from impurities exist. Ingestion of grape pomace extract from its intended use results in levels of intake of grape pomace extract and its constituents that are within safe limits established by published animal toxicity studies and human studies of grape seed and grape pomace extract and their constituents as well as experience from the history of safe consumption of foods containing these constituents. Therefore, the intended use of exGrape® Total grape pomace extract, produced and used in accordance with cGMP and complying with the specifications described in this GRAS monograph, is safe and Generally Recognized As Safe (GRAS) based on scientific procedures.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusion. Therefore, the intended use of exGrape® Total grape pomace extract is safe, and is GRAS, via scientific procedures.

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Signature: [redacted] Date: 30 July 2012

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APPENDIX
Analyses of Pesticide Residues

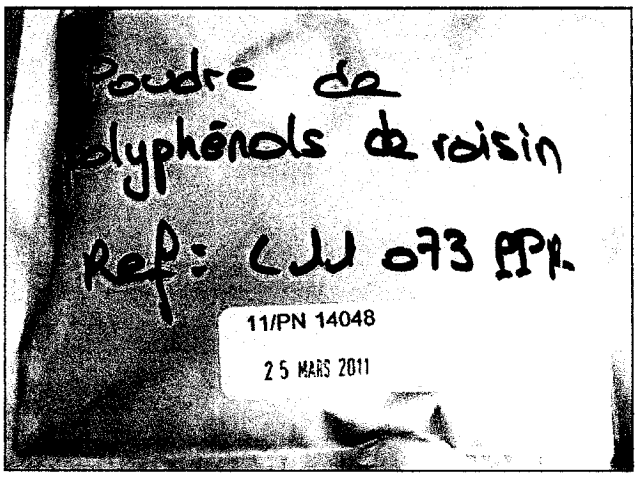
Phytocontrol
Laboratoire d'analyses phytosanitaires

RAPPORT D'ANALYSES N°R1114048_V0
DATE : 06/04/2011

L UNION GRAP SUD
A l'attention de Mme Nelly URBAN
30360 CRUVIERS LASCOURS

Notre référence	11/PN14048
Votre référence	L11 073 PPR
Nature de l'échantillon	Poudre de polyphénols de raisin
Date de réception	25/03/2011
Echantillonnage	Client
Transport	La Poste
Référence de devis	DNI110021
Analyse demandée	
Pesticides	Multirésidus GC 250 + Multirésidus LC 150 Dithiocarbamates

Echantillon à réception



Résultats d'analyses

	Résultat	Unité	LQ	LMR	Fin d'analyse
Pesticides					
Multirésidus GC 250					
Benalaxyl dont Benalaxyl-M	0.11	mg/kg	0,01	(2)	06/04/2011
Fenhexamide	0.16	mg/kg	0,01	(1)	06/04/2011
Fludioxonil	0.057	mg/kg	0,01	(2)	06/04/2011
Iprodione	0.41	mg/kg	0,01	(1)	06/04/2011
Phtalimide	33	mg/kg	0,01	(2)	06/04/2011
Procymidone	0.054	mg/kg	0,01	(1)	06/04/2011
Pyrimethanil	0.29	mg/kg	0,01	(2)	06/04/2011
Tebuconazole	0.29	mg/kg	0,01	(1)	06/04/2011
Multirésidus LC 150					
Azoxystrobine	0.058	mg/kg	0,01	(2)	30/03/2011
Carbendazim (+Benomyl)	0.19	mg/kg	0,01	(2)	30/03/2011
Dimethomorphe	1.1	mg/kg	0,01	(1)	30/03/2011
Iprovalicarb	0.81	mg/kg	0,01	(2)	30/03/2011
Metrafenone	0.036	mg/kg	0,01	(2)	30/03/2011
Spiroxamine	0.30	mg/kg	0,01	(2)	30/03/2011
Tebufenozide	0.21	mg/kg	0,01	(2)	30/03/2011
Tetraconazole	0.058	mg/kg	0,01	(1)	30/03/2011
Trifloxystrobine	0.018	mg/kg	0,01	(2)	30/03/2011
Monorésidus spécifiques					
Dithiocarbamates (CS2)	0.070	mg/kg	0,05		29/03/2011

Détail des paramètres analysés et des méthodes utilisées en page(s) suivante(s)

Légende

ND = Non détecté D = Détecté LQ = Limite de Quantification LMR = Limite Maximale de Résidu autorisée (sur produit frais).
Note : les valeurs de référence prise en compte pour les analyses des résidus de pesticides sont issues du règlement (CE) n°149/2008 de la Commission du 29 Janvier 2008.
Ce texte établit les LMR applicables sur le marché de l'UE, par l'entrée en vigueur du règlement (CE) n°396/2005 du Parlement Européen et du Conseil, seule législation désormais applicable.
Méthodes utilisées mentionnées en page(s) suivante(s) :

MOC3/05 version 0 : Détermination de la teneur en résidus de pesticides par GC-MS(n) et/ou LC-MS-MS : méthode interne.

MOC3/11 version 0 : Détermination des résidus de dithiocarbamates dans les fruits et légumes par GC-MS/HS : méthode interne.

Phytocontrol
Laboratoire d'analyses phytosanitaires

Commentaires

(1) Famille des organo-chlorés et organo-phosphoré
(2) Famille des organo-azotés et divers.
Benalaxyl dont Benalaxyl-M : somme des isomères
Carbendazim (+Benomyl) : somme du benomyl et du carbendazim, exprimée en carbendazim
Dithiocarbamates (CS2) : y compris manèbe, mancozèbe, métirame, propinèbe, thirame et zirame
Phtalimide : Le phtalimide est un métabolite non règlementé du Folpet. Selon le règlement 396/2005, le calcul de la LMR du Folpet ne tient pas compte de son métabolite et aucune LMR n'est définie pour le phtalimide.

Signature

Rapport validé par :

Céline TAFFIN
Réglementation et Sécurité Alimentaire

Eric CAPODANNO
Directeur Scientifique

Pesticides

Multirésidus GC 250

FB3/02.c vers. 4 du 01/02/2011

Unité : mg/kg	Résultat	LQ	Méthode
2-Phenylphenol	ND	0,01	MOC3/05
Acephate	ND	0,01	MOC3/05
Acetochlor	ND	0,01	MOC3/05
Acibenzolar-S-methyl	ND	0,01	MOC3/05
Aclonifen	ND	0,01	MOC3/05
Acrinathrine	ND	0,01	MOC3/05
Alachlore	ND	0,01	MOC3/05
Ametryn	ND	0,01	MOC3/05
Anthraquinone	ND	0,01	MOC3/05
Atrazine	ND	0,01	MOC3/05
Azinphos-methyl	ND	0,01	MOC3/05
Benalaxyl dont Benalaxyl-M	0.11	0,01	MOC3/05
Bendiocarb	ND	0,01	MOC3/05
Benfluraline	ND	0,01	MOC3/05
Benoxacor	ND	0,01	MOC3/05
Bentazone	ND	0,01	MOC3/05
Bifenox	ND	0,01	MOC3/05
Bifenthrine	ND	0,01	MOC3/05
Biphenyl	ND	0,01	MOC3/05
Bitertanol	ND	0,01	MOC3/05
Bromacil	ND	0,01	MOC3/05
Bromophos-ethyl	ND	0,01	MOC3/05
Bromophos-methyl	ND	0,01	MOC3/05
Bromopropylate	ND	0,01	MOC3/05
Bupirimate	ND	0,01	MOC3/05
Buprofezin	ND	0,01	MOC3/05
Butralin	ND	0,01	MOC3/05
Cadusaphos	ND	0,01	MOC3/05
Captafol	ND	0,01	MOC3/05
Captan	ND	0,01	MOC3/05
Carbaryl	ND	0,01	MOC3/05
Carbofenothion	ND	0,01	MOC3/05
Carbofuran (+3-Hydroxy)	ND	0,01	MOC3/05
Carfentrazone-ethyl	ND	0,01	MOC3/05
Chlorbenside	ND	0,01	MOC3/05
Chlordane (cis + trans)	ND	0,01	MOC3/05
Chlorfenson	ND	0,01	MOC3/05
Chlorfenvinphos	ND	0,01	MOC3/05
Chlorobenzilate	ND	0,01	MOC3/05
Chlorothalonil	ND	0,01	MOC3/05
Chlorpropham(+3Chloroanilin)	ND	0,01	MOC3/05
Chlorpyrifos	ND	0,01	MOC3/05
Chlorpyrifos-methyl	ND	0,01	MOC3/05
Chlorthal dimethyl	ND	0,01	MOC3/05
Chlorthiophos	ND	0,01	MOC3/05
Chlozolinate	ND	0,01	MOC3/05
clodinafop p. ester	ND	0,01	MOC3/05
Clomazone	ND	0,01	MOC3/05
Coumaphos	ND	0,01	MOC3/05
Cyfluthrine (β+γ)	ND	0,01	MOC3/05
Cyhalofop-butyl	ND	0,01	MOC3/05
Cyhalothrine (lambda)	ND	0,01	MOC3/05
Cypermethrine (α+β+θ+ζ)	ND	0,01	MOC3/05
Cyproconazole	ND	0,01	MOC3/05
Cyprodinil	ND	0,01	MOC3/05
DDT (Σ des isomères)	ND	0,01	MOC3/05
Deltamethrine	ND	0,01	MOC3/05
Demeton-S	ND	0,01	MOC3/05
Demeton-S-methyl	ND	0,01	MOC3/05
Dialifos	ND	0,01	MOC3/05
Diallate	ND	0,01	MOC3/05
Diazinon	ND	0,01	MOC3/05
Dichlobenil	ND	0,01	MOC3/05
Dichlofenthion	ND	0,01	MOC3/05
Dichlofluanide	ND	0,01	MOC3/05
Dichlorvos	ND	0,01	MOC3/05
Diclofop-methyl	ND	0,01	MOC3/05
Dicofol (Σ des isomères)	ND	0,01	MOC3/05
Dieldrin (+Aldrin)	ND	0,01	MOC3/05
Diethofencarb	ND	0,01	MOC3/05
Difenoconazole	ND	0,01	MOC3/05
Diflufenican	ND	0,01	MOC3/05
Dimetachlor	ND	0,01	MOC3/05
Dimethoate (+Omethoate)	ND	0,01	MOC3/05
Diphenylamine	ND	0,01	MOC3/05
Disulfoton	ND	0,01	MOC3/05
Ditalimfos	ND	0,01	MOC3/05
Edifenphos	ND	0,01	MOC3/05
Endosulfan (α+β+sulfate)	ND	0,01	MOC3/05
Endrin	ND	0,01	MOC3/05
EPTC	ND	0,01	MOC3/05
Ethiofencarb	ND	0,01	MOC3/05
Ethion	ND	0,01	MOC3/05
Ethofumesate	ND	0,01	MOC3/05
Ethoprophos	ND	0,01	MOC3/05
Ethoxyquin	ND	0,01	MOC3/05
Etofenprox	ND	0,01	MOC3/05
Etridiazole	ND	0,01	MOC3/05
Etrimphos	ND	0,01	MOC3/05
Famoxadone	ND	0,01	MOC3/05
Famphur	ND	0,01	MOC3/05
Fempropathrine	ND	0,01	MOC3/05
Fenamiphos	ND	0,01	MOC3/05
Fenarimol	ND	0,01	MOC3/05
Fenazaquin	ND	0,01	MOC3/05
Fenbuconazole	ND	0,01	MOC3/05
Fenchlorphos (+oxon)	ND	0,01	MOC3/05
Fenhexamide	0.16	0,01	MOC3/05
Fenitrothion	ND	0,01	MOC3/05
Fenoxaprop-ethyl	ND	0,01	MOC3/05
Fenoxycarbe	ND	0,01	MOC3/05
Fenpropidine	ND	0,01	MOC3/05
Fenpropimorphe	ND	0,01	MOC3/05
Fenson	ND	0,01	MOC3/05
Fensulfothion (+sulfone)	ND	0,01	MOC3/05
Fenthion(+sulfone+sulfoxide)	ND	0,01	MOC3/05
Fenvalerate (RR + SS)	ND	0,01	MOC3/05
Fenvalerate (RS + SR)	ND	0,01	MOC3/05
Fipronil (+sulfone)	ND	0,01	MOC3/05
Fluazifop p butyl	ND	0,01	MOC3/05
Fluazinam	ND	0,01	MOC3/05
Fludioxonil	0.057	0,01	MOC3/05
Flufenacet	ND	0,01	MOC3/05
Flurochloridone	ND	0,01	MOC3/05
Fluroxypyr-methylhexyl ester	ND	0,01	MOC3/05
Flusilazole	ND	0,01	MOC3/05
Flutolanil	ND	0,01	MOC3/05
Flutriafol	ND	0,01	MOC3/05
Fluvalinate (Tau)	ND	0,01	MOC3/05
Folpet	ND	0,01	MOC3/05
Fonofos	ND	0,01	MOC3/05
Formothion	ND	0,01	MOC3/05
Fosthiazate	ND	0,01	MOC3/05
Furalaxyl	ND	0,01	MOC3/05
Furathiocarb	ND	0,01	MOC3/05
HCB	ND	0,01	MOC3/05
HCH (α+β+δ)	ND	0,01	MOC3/05
HCH gamma	ND	0,01	MOC3/05
Heptachlore (+epoxyde)	ND	0,01	MOC3/05
Heptenophos	ND	0,01	MOC3/05
Hexaconazole	ND	0,01	MOC3/05
Hexazinone	ND	0,01	MOC3/05
Imazalil	ND	0,01	MOC3/05
Iodofenphos	ND	0,01	MOC3/05
Iprodione	0.41	0,01	MOC3/05
Isofenphos-ethyl	ND	0,01	MOC3/05
Isofenphos-methyl	ND	0,01	MOC3/05
Isoxaben	ND	0,01	MOC3/05
Isoxaflutole	ND	0,01	MOC3/05
Malathion (+Malaoxon)	ND	0,01	MOC3/05
Mecarbam	ND	0,01	MOC3/05
Mepanipyrim	ND	0,01	MOC3/05
Mepronil	ND	0,01	MOC3/05
Metalaxyl dont Metalaxyl-M	ND	0,01	MOC3/05
Metazachlor	ND	0,01	MOC3/05
Methacrifos	ND	0,01	MOC3/05
Methamidophos	ND	0,01	MOC3/05
Methidathion	ND	0,01	MOC3/05
Methiocarb (+sulfone)	ND	0,01	MOC3/05
Methoxychlor	ND	0,01	MOC3/05
Metolachlor dont S-Metolachlor	ND	0,01	MOC3/05
Metribuzine	ND	0,01	MOC3/05
Mevinphos	ND	0,01	MOC3/05
Mirex	ND	0,01	MOC3/05
Molinate	ND	0,01	MOC3/05
Monalide	ND	0,01	MOC3/05
Monocrotophos	ND	0,01	MOC3/05
Myclobutanil	ND	0,01	MOC3/05
Napropamide	ND	0,01	MOC3/05
Nitrofen	ND	0,01	MOC3/05
Nitrothal isopropyle	ND	0,01	MOC3/05
Norflurazon	ND	0,01	MOC3/05
Nuarimol	ND	0,01	MOC3/05
Oxadiazon	ND	0,01	MOC3/05
Oxadixyl	ND	0,01	MOC3/05
Oxyfluorfen	ND	0,01	MOC3/05
Parathion-ethyl	ND	0,01	MOC3/05
Parathion-methyl	ND	0,01	MOC3/05
PCB 028	ND	0,01	MOC3/05
PCB 052	ND	0,01	MOC3/05
PCB 101	ND	0,01	MOC3/05
PCB 118	ND	0,01	MOC3/05
PCB 138	ND	0,01	MOC3/05
PCB 153	ND	0,01	MOC3/05
PCB 180	ND	0,01	MOC3/05
Penconazole	ND	0,01	MOC3/05
Pendimethaline	ND	0,01	MOC3/05
Pentachloroanisole	ND	0,01	MOC3/05
Permethrine (cis + trans)	ND	0,01	MOC3/05
Perthane	ND	0,01	MOC3/05
Phenothrine	ND	0,01	MOC3/05
Phenthoate	ND	0,01	MOC3/05
Phorate (+sulfone)	ND	0,01	MOC3/05
Phosalone	ND	0,01	MOC3/05

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SARL au capital de 30.000 euros - RCS Nîmes 490 024 049 - TVA intra FR 08 490 024 049 - APE 7120B

Phtalimide	33	0,01	MOC3/05
Piperonyl butoxide	ND	0,01	MOC3/05
Pirimicarb (+desmethyl)	ND	0,01	MOC3/05
Pirimiphos-ethyl	ND	0,01	MOC3/05
Pirimiphos-methyl	ND	0,01	MOC3/05
Pretilachlore	ND	0,01	MOC3/05
Prochloraz (+TCP)	ND	0,01	MOC3/05
Procymidone	0.054	0,01	MOC3/05
Profenophos	ND	0,01	MOC3/05
Profluralin	ND	0,01	MOC3/05
Prometon	ND	0,01	MOC3/05
Prometryn	ND	0,01	MOC3/05
Propachlor	ND	0,01	MOC3/05
Propamocarb	ND	0,01	MOC3/05
Propargite	ND	0,01	MOC3/05
Propazine	ND	0,01	MOC3/05
Propetamphos	ND	0,01	MOC3/05
Propham	ND	0,01	MOC3/05
Propiconazole	ND	0,01	MOC3/05
Propyzamide	ND	0,01	MOC3/05
Proquinazid	ND	0,01	MOC3/05
Prosulfocarb	ND	0,01	MOC3/05
Prothiophos	ND	0,01	MOC3/05
Prothoate	ND	0,01	MOC3/05
Pyrazophos	ND	0,01	MOC3/05
Pyridaben	ND	0,01	MOC3/05
Pyridaphenthion	ND	0,01	MOC3/05
Pyrifenox	ND	0,01	MOC3/05
Pyrimethanil	0.29	0,01	MOC3/05
Pyriproxyfen	ND	0,01	MOC3/05
Quinalphos	ND	0,01	MOC3/05
Quinomethionate	ND	0,01	MOC3/05
Quinoxyfen	ND	0,01	MOC3/05
Quintozene (+ PCNB+MPCPS)	ND	0,01	MOC3/05
Quizalofop-ethyl	ND	0,01	MOC3/05
Resmethrine	ND	0,01	MOC3/05
Sectbumeton	ND	0,01	MOC3/05
Sulfotep	ND	0,01	MOC3/05
Sulprofos	ND	0,01	MOC3/05
Tebuconazole	0.29	0,01	MOC3/05
Tebufenpyrad	ND	0,01	MOC3/05
Tebutam	ND	0,01	MOC3/05
Tecnazene	ND	0,01	MOC3/05
Tefluthrine	ND	0,01	MOC3/05
Terbacil	ND	0,01	MOC3/05
terbufos	ND	0,01	MOC3/05
Terbuthylazine	ND	0,01	MOC3/05
Terbutryne	ND	0,01	MOC3/05
Tetrachlorvinphos	ND	0,01	MOC3/05
Tetradifon	ND	0,01	MOC3/05
Tetrahydrophtalimide	ND	0,01	MOC3/05
Tetramethrine	ND	0,01	MOC3/05
Thiabendazole	ND	0,01	MOC3/05
Thiometon	ND	0,01	MOC3/05
Tolclofos-methyl	ND	0,01	MOC3/05
Tolylfluanid	ND	0,01	MOC3/05
Tralomethrine	ND	0,01	MOC3/05
Transfluthrin	ND	0,01	MOC3/05
Triadimefon + Triadimenol	ND	0,01	MOC3/05
Triallate	ND	0,01	MOC3/05
Triamiphos	ND	0,01	MOC3/05
Triazophos	ND	0,01	MOC3/05
Trichloronat	ND	0,01	MOC3/05
Trifluraline	ND	0,01	MOC3/05
Vinclozoline(+3,5dichloroanilin)	ND	0,01	MOC3/05
Zoxamide	ND	0,01	MOC3/05

Multirésidus LC 150
FB3/02.d vers. 3 du 15/10/2010

Unité : mg/kg	Résulat	LQ	Méthode
1-naphtyl acetamide	ND	0,01	MOC3/05
Acetamipride	ND	0,01	MOC3/05
Aldicarb (+sulfoxide)	ND	0,01	MOC3/05
Amitraze (+2,4 dimethylaniline)	ND	0,01	MOC3/05
Atrazine desethyl (+déisopropyl)	ND	0,01	MOC3/05
Azaconazole	ND	0,01	MOC3/05
Azimsulfuron	ND	0,01	MOC3/05
Azinphos-ethyl	ND	0,01	MOC3/05
Azoxystrobine	0.058	0,01	MOC3/05
Benfuracarb	ND	0,01	MOC3/05
Bensulfuron-methyl	ND	0,01	MOC3/05
Benthiavalicarb-isopropyl	ND	0,01	MOC3/05
Bifenazate	ND	0,01	MOC3/05
Bispyribac-Sodium	ND	0,01	MOC3/05
Boscalide	ND	0,01	MOC3/05
Bromuconazole	ND	0,01	MOC3/05
Butafenacil	ND	0,01	MOC3/05
Butoxycarboxim	ND	0,01	MOC3/05
Buturon	ND	0,01	MOC3/05
Carbendazim (+Benomyl)	0.19	0,01	MOC3/05
Carbetamide	ND	0,01	MOC3/05
Carbosulfan	ND	0,01	MOC3/05
Carboxin	ND	0,01	MOC3/05
Chloridazon	ND	0,01	MOC3/05
Chloroxuron	ND	0,01	MOC3/05
Chlorthiamid	ND	0,01	MOC3/05
Chlortoluron	ND	0,01	MOC3/05
Cinosulfuron	ND	0,01	MOC3/05
Clethodim + Sethoxydim	ND	0,01	MOC3/05
Clofentezine	ND	0,01	MOC3/05
Cloquintocet 1methylhexyl ester	ND	0,01	MOC3/05
Cyanazine	ND	0,01	MOC3/05
Cyazofamide	ND	0,01	MOC3/05
Cycloxydime	ND	0,01	MOC3/05
Cycluron	ND	0,01	MOC3/05
Demeton-S-methyl sulfone	ND	0,01	MOC3/05
Desmedipham	ND	0,01	MOC3/05
Desmetryn	ND	0,01	MOC3/05
Diafenthiuron	ND	0,01	MOC3/05
Diclobutrazol	ND	0,01	MOC3/05
Difenacoum	ND	0,01	MOC3/05
Dimethenamid-P	ND	0,01	MOC3/05
Dimethomorphe	1.1	0,01	MOC3/05
Diniconazole	ND	0,01	MOC3/05
Disulfoton-sulfone	ND	0,01	MOC3/05
Diuron	ND	0,01	MOC3/05
DMST	ND	0,01	MOC3/05
Dodine	ND	0,01	MOC3/05
Emamectin benzoate	ND	0,01	MOC3/05
Epoxyconazole	ND	0,01	MOC3/05
Ethidimuron	ND	0,01	MOC3/05
Etoxazole	ND	0,01	MOC3/05
Fenamidone	ND	0,01	MOC3/05
Fenamiphos-sulfone(+sulfoxide)	ND	0,01	MOC3/05
Fenpiroximate	ND	0,01	MOC3/05
Fensulfothion-oxon (+sulfone)	ND	0,01	MOC3/05
Fenthion-oxon(+sulfone+sulfox.)	ND	0,01	MOC3/05
Fenuron	ND	0,01	MOC3/05
Flazasulfuron	ND	0,01	MOC3/05
Flonicamid	ND	0,01	MOC3/05
Flufenoxuron	ND	0,01	MOC3/05
Fluometuron	ND	0,01	MOC3/05
Fluoxastrobin	ND	0,01	MOC3/05
Fluquinconazole	ND	0,01	MOC3/05
Flurtamone	ND	0,01	MOC3/05
Fluthiacet-methyl	ND	0,01	MOC3/05
Fomesafen	ND	0,01	MOC3/05
Foramsulfuron	ND	0,01	MOC3/05
Forchlofenuron	ND	0,01	MOC3/05
Formetanate (hydrochloride)	ND	0,01	MOC3/05
Fuberidazole	ND	0,01	MOC3/05
Halosulfuron-methyl	ND	0,01	MOC3/05
Hexythiazox	ND	0,01	MOC3/05
Hydramethylnon	ND	0,01	MOC3/05
Imazaquin	ND	0,01	MOC3/05
Imidachlopride	ND	0,01	MOC3/05
Indoxacarb	ND	0,01	MOC3/05
Iprovalicarb	0.81	0,01	MOC3/05
Isopropaline	ND	0,01	MOC3/05
Isoprothiolane	ND	0,01	MOC3/05
Isoproturon	ND	0,01	MOC3/05
Isoxathion	ND	0,01	MOC3/05
Kresoxim-methyl	ND	0,01	MOC3/05
Lenacil	ND	0,01	MOC3/05
Linuron	ND	0,01	MOC3/05
Lufenuron	ND	0,01	MOC3/05
Mandipropamide	ND	0,01	MOC3/05
Mesosulfuron methyl	ND	0,01	MOC3/05
Metamitron	ND	0,01	MOC3/05
Metconazole	ND	0,01	MOC3/05
Methabenzthiazuron	ND	0,01	MOC3/05
Methiocarb-sulfoxide	ND	0,01	MOC3/05
Methomyl + Thiodicarb	ND	0,01	MOC3/05
Methoxyfenozide	ND	0,01	MOC3/05
Metobromuron	ND	0,01	MOC3/05
Metoxuron	ND	0,01	MOC3/05
Metrafenone	0.036	0,01	MOC3/05
Metsulfuron-methyl	ND	0,01	MOC3/05
Monolinuron	ND	0,01	MOC3/05
Monuron	ND	0,01	MOC3/05
Neburon	ND	0,01	MOC3/05
Nicosulfuron	ND	0,01	MOC3/05
Novaluron	ND	0,01	MOC3/05
Oxamyl	ND	0,01	MOC3/05
Oxasulfuron	ND	0,01	MOC3/05
Paclobutrazol	ND	0,01	MOC3/05
Paraoxon-ethyl	ND	0,01	MOC3/05
Pencycuron	ND	0,01	MOC3/05
Phenmedipham	ND	0,01	MOC3/05
Phosmet (+oxon)	ND	0,01	MOC3/05
Phosphamidon	ND	0,01	MOC3/05
Phoxim	ND	0,01	MOC3/05
Picolinafen	ND	0,01	MOC3/05
Picoxystrobine	ND	0,01	MOC3/05
Pinoxadene	ND	0,01	MOC3/05
Propanil	ND	0,01	MOC3/05
Propaquizafop	ND	0,01	MOC3/05
Propoxur	ND	0,01	MOC3/05
Prosulfuron	ND	0,01	MOC3/05
Prothioconazole (+desthio)	ND	0,01	MOC3/05

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Pyraclostrobine	ND	0,01	MOC3/05
Pyraflufen-ethyl	ND	0,01	MOC3/05
Pyridate	ND	0,01	MOC3/05
Rotenone	ND	0,01	MOC3/05
Sebuthylazine	ND	0,01	MOC3/05
Simazine	ND	0,01	MOC3/05
Spinosad	ND	0,01	MOC3/05
Spirodiclofen	ND	0,01	MOC3/05
Spiromesifen	ND	0,01	MOC3/05
Spiroxamine	0.30	0,01	MOC3/05
Sulfosulfuron	ND	0,01	MOC3/05
TCMTB	ND	0,01	MOC3/05
Tebufenozide	0.21	0,01	MOC3/05
Tepraloxydim	ND	0,01	MOC3/05
Terbufos-sulfoxide (+sulfone)	ND	0,01	MOC3/05
Terbumeton	ND	0,01	MOC3/05
Tetraconazole	0.058	0,01	MOC3/05
Thiachloprid	ND	0,01	MOC3/05
Thiamethoxam (+Clothianidine)	ND	0,01	MOC3/05
Thiophanate-methyl	ND	0,01	MOC3/05
Triazamate	ND	0,01	MOC3/05
Tricyclazole	ND	0,01	MOC3/05
Tridemorphe	ND	0,01	MOC3/05
Trifloxystrobine	0.018	0,01	MOC3/05
Trifloxysulfuron	ND	0,01	MOC3/05
Triflumizole	ND	0,01	MOC3/05
Triflusulfuron-methyl	ND	0,01	MOC3/05
Triforine	ND	0,01	MOC3/05
Triticonazole	ND	0,01	MOC3/05
Warfarin	ND	0,01	MOC3/05

Monorésidus spécifiques

Unité : mg/kg	Résulat	LQ	Méthode
Dithiocarbamates (CS2)	0.070	0,05	MOC3/11

Phytocontrol
Laboratoire d'analyses phytosanitaires

RAPPORT D'ANALYSES N°R101169_V0
DATE : 04/03/2010

L UNION GRAP SUD
A l'attention de Mme Nelly URBAN
30360 CRUVIERS LASCOURS

Notre référence	10/PN1169
Votre référence	L10 060 PPR
Nature de l'échantillon	Poudre de polyphenols de raisin
Date de réception	01/03/2010
Echantillonnage	Client
Transport	Colissimo
Référence de devis	DPN090265
Analyse demandée	
Pesticides	Dithiocarbamates
	Multirésidus GC 250 + Multirésidus LC 150

Echantillon à réception

BEST ORIGINAL COPY

000087.001

Résultats d'analyses

	Résultat	Unité	LQ	LMR	Fin d'analyse
Pesticides					
Multirésidus GC 250					
Tebuconazole	0.21	mg/kg	0.01	(1)	03/03/2010
Iprodione	1.6	mg/kg	0.01	(1)	03/03/2010
Fludioxonil	0.060	mg/kg	0.01	(2)	03/03/2010
Fenhexamide	0.83	mg/kg	0.01	(1)	03/03/2010
Cyprodinil	0.048	mg/kg	0.01	(2)	03/03/2010
Multirésidus LC 150					
Trifloxystrobine	D < 0.01	mg/kg	0.01	(2)	03/03/2010
Spiroxamine	0.16	mg/kg	0.01	(2)	03/03/2010
Metrafenone	0.028	mg/kg	0.01	(2)	03/03/2010
Iprovalicarb	0.54	mg/kg	0.01	(2)	03/03/2010
Dimethomorphe	0.68	mg/kg	0.01	(1)	03/03/2010
Carbendazim	0.16	mg/kg	0.01	(2)	03/03/2010
Boscalide	0.91	mg/kg	0.01	(1)	03/03/2010
Azoxystrobine	0.035	mg/kg	0.01	(2)	03/03/2010
Monorésidus spécifiques					
Dithiocarbamates (CS2)	0.063	mg/kg	0.05		04/03/2010

Détail des paramètres analysés et des méthodes utilisées en page(s) suivante(s)

Légende

ND = Non détecté D = Détecté LQ = Limite de Quantification LMR = Limite Maximale de Résidu autorisée (sur produit frais).

Note : les valeurs de référence prise en compte pour les analyses des résidus de pesticides sont issues du règlement (CE) n°149/2008 de la Commission du 29 Janvier 2008. Ce texte établit les LMR applicables sur le marché de l'UE, par l'entrée en vigueur du règlement (CE) n° 396/2005 du Parlement Européen et du Conseil, seule législation désormais applicable.

Méthodes utilisées mentionnées en page(s) suivante(s) :

MOC3/05 version 0 : Détermination de la teneur en résidus de pesticides par GC-MS et/ou GC-MS-MS et/ou LC-MS-MS : méthode interne.

MOC3/11 version 0 : Détermination des résidus de dithiocarbamates par GC-MS : méthode interne.

Commentaires

(1) Famille des organo-chlorés et organo-phosphorés
(2) Famille des organo-azotés et divers.
Carbendazim : somme avec benomyl

Signature

Rapport validé par le Directeur Technique
Eric CAPODANNO

Sethoxydim	ND	0,01	MOC3/05
Simazine	ND	0,01	MOC3/05
Spinosad	ND	0,01	MOC3/05
Spirodiclofen	ND	0,01	MOC3/05
Spiromesifen	ND	0,01	MOC3/05
Spirotetramine	0,16	0,01	MOC3/05
Sulfosulfuron	ND	0,01	MOC3/05
TCMTB (busan)	ND	0,01	MOC3/05
Tebufenozide	ND	0,01	MOC3/05
Tepraloxydim	ND	0,01	MOC3/05
Terbufos-sulfoxide (+ sulfone)	ND	0,01	MOC3/05
Terbumeton	ND	0,01	MOC3/05
Tetraconazole	ND	0,01	MOC3/05
Thiachloprid	ND	0,01	MOC3/05
Thiodicarb	ND	0,01	MOC3/05
Thiophanate-methyl	ND	0,01	MOC3/05
Triazamate	ND	0,01	MOC3/05
Tricyclazole	ND	0,01	MOC3/05
Tridemorphe	ND	0,01	MOC3/05
Trifloxystrobine	D < 0,01	0,01	MOC3/05
Trifloxysulfuron	ND	0,01	MOC3/05
Triflumizole	ND	0,01	MOC3/05
Triflusulfuron-methyl	ND	0,01	MOC3/05
Triforine	ND	0,01	MOC3/05
Triticonazole	ND	0,01	MOC3/05
Warfarin	ND	0,01	MOC3/05

Monorésidus spécifiques

Unité : mg/kg	Résulat	LQ	Méthode
Dithiocarbamates (CS2)	0,063	0,05	MOC3/11

SUBMISSION END